Lake remediation by top-down and bottom-up management: An ecosystem-scale experiment in the English Lake District

Amy M. Anderson BSc. (Hons.)

Lancaster Environment Centre
Faculty of Science and Technology
Lancaster University

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Abstract

Lake ecosystems face a variety of pressures arising from catchment-based anthropogenic activities and long-term changes in background environmental conditions, which greatly threaten their structure, function and the sustainable provision of essential ecosystem services. The response of lakes to external perturbations is controlled by a complex interaction of top-down (predation) and bottom-up (resource availability) food web processes. A better understanding of how these processes interact is vital if they are to be effectively manipulated in the remediation of degraded lakes. In this study, Esthwaite Water, a eutrophic lake in the English Lake District, was used as the model ecosystem to investigate the relative importance of these two opposing forces in determining water quality changes in response to anthropogenic pressures, specifically eutrophication and climate change.

The research is presented as three studies which address the historical, contemporary and future impact of stressors on the ecological structure and function of Esthwaite Water. Analysis of long-term monitoring data determined that phosphorus availability was the main driver of water quality degradation throughout the study period (1970 to 2014). Despite substantial reductions to the external phosphorus load in later decades, water quality improvements were not observed. Phosphorus release from lake sediments and the effects of climate change on phytoplankton community structure appear to have buffered against the desired impact of reduced external nutrient loads. Analysis of contemporary surface sediments revealed a legacy of phosphorus enrichment from years of high external loading, including from aquaculture cages previously installed on the lake. Much of the sediment phosphorus was determined to be stored in release-sensitive forms.

The PROTECH lake model was used to test the response of the phytoplankton community to variations in both nutrient availability and zooplankton grazing rate. The dominant controlling factor on total phytoplankton biomass was nutrient availability. When nutrient loads were high, cyanobacteria dominated the phytoplankton community. Such conditions limited the potential for top-down regulation by herbivory owing to food quality constraints upon zooplankton. The inclusion of climate warming in modelling scenarios resulted in the exacerbation of the symptoms of eutrophication. As such, it is recommended that future attempts at water quality improvement take a bottom-up approach, with a focus on controlling the internal phosphorus load.

Declaration

I declare that the thesis presented here is the result of my own independent work, except where stated otherwise by reference or acknowledgment. It has not previously been submitted for the award of a higher degree at another university or institution.

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Amy M. Anderson

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Table of Contents

List of Figures

List of Tables

Notation

A	Area
AEP	Alkali-extractable phosphorus
AIC	Akaike information criteria
AICc	Second-order Akaike information criteria
ANOVA	Analysis of variance
BACI	Before-after-control-impact
BB	Black Beck
CEH	Centre for Ecology & Hydrology
Chl	Chlorophyll *a*
D	Phytoplankton loss rate due to dilution
Dep	Depth
d.f.	Degrees of freedom
DO	Dissolved oxygen
DW	Dry weight
ELA	Experimental Lakes Area
Est.	Estimate
F	F-statistic
FBA	Freshwater Biological Association
FF	Fish food
G	Phytoplankton loss rate due to grazing
GAM	Generalized additive model
GLM	Generalized linear model
GM	Geometric mean
GPS	Global Positioning System
IPCC	Intergovernmental Panel on Climate Change
K	Conversion factor (per second to per month)
K_d	Eddy diffusivity
LMM	Linear mixed-effects model
LOD	Limit of detection
Mean Sq.	Mean of squares
M_{TP}	Mass of total phosphorus
n	Sample size
NAO	North Atlantic Oscillation
NRFA	National River Flow Archive

p	p value
PEG	Phytoplankton Ecology Group
PROTECH	Phytoplankton Responses to Environmental Change model
Q	Discharge
r	Pearson product-moment correlation coefficient
r'	Phytoplankton growth rate
RWB	Rossby wave breaking
S	Phytoplankton loss rate due to settling
SD	Standard deviation
SRP	Soluble reactive phosphorus
St. Err.	Standard error
Sum Sq.	Sum of squares
t	t-value
T	Time
TP	Total phosphorus
UK	United Kingdom
V	Volume
WFD	Water Framework Directive
WHO	World Health Organisation
WW	Wet weight
WwTW	Wastewater treatment works

The International System of Units was used throughout

Chapter One

General Introduction

1.1 Overview

Although they only contain 0.3 % of all the world's water (Shiklomanov & Rodda 2003), freshwater ecosystems are disproportionality important for humanity and key contributors to global biodiversity. Approximately 77 % of the liquid surface freshwater on Earth is contained in natural and artificial lakes (Shiklomanov 1993). Lakes cover more than 4.2 million km^2 worldwide, and in the United Kingdom (UK) there are more than 43,000 lakes and ponds with a surface area greater than one hectare (Hughes *et al.* 2004). As a habitat for a wide range of plant and animal species, lakes are a valuable source of biodiversity at a local, regional and national scale (Dudgeon *et al.* 2006; Moss *et al.* 2009). Freshwater lakes also provide numerous essential provisioning, regulating, supporting and cultural ecosystem services upon which humankind depends. Direct contributions of lake ecosystems to human well-being and way of life include, but are by no means limited to: water for domestic and industrial purposes, irrigation, fisheries, transport of commercial goods, flood regulation, energy generation, recreation and tourism (Maltby & Ormerod 2011; Millennium Ecosystem Assessment 2005; Wilson & Carpenter 1999). Indirectly, lakes support our survival by regulating the biogeochemical cycle of carbon, and consequently global climate change (Cole *et al.* 2007; Maberly *et al.* 2013; Tranvik *et al.* 2009). An assessment by Costanza *et al*. (2014) estimated the combined economic value of ecosystem services provided by lakes and rivers at US$ 2.5 trillion per year globally.

Unfortunately, lakes are subject to multiple stressors arising from anthropogenic activities and from inter-annual and long-term background changes in environmental conditions, which can affect ecological and chemical status and ultimately jeopardise the provision of ecosystem services (Folt *et al.* 1999; Heathwaite 2010; Navarro-Ortega *et al.* 2015; Ormerod *et al.* 2010). Lakes are especially sensitive to environmental perturbations given their high connectivity to surrounding landscapes and the atmosphere (Maberly & Elliott 2012; Schindler 2009a). Nutrient enrichment and siltation caused by agricultural practices, the effect of hydropower plants on catchment hydrology, acidification, habitat degradation, introduction of non-native species, over exploitation of resources, flooding and drought are all long-standing stressors throughout Europe (Hering *et al.* 2015; Ormerod *et al.* 2010). Over the last decade, these established threats to lake ecosystems have intensified and evolved, and new or previously unrecognised threats have become more apparent. For example, changing climates (Moss *et al.* 2009), pharmaceuticals (Chèvre 2014), microplastic pollution (Free *et al.* 2014), engineered nanoparticles (Gottschalk *et al.* 2013) and freshwater salinisation (Herbert *et al.* 2015). Stressors do not

usually act independently, but rather interact to produce combined impacts on biodiversity and ecosystem functioning (Craig *et al.* 2017; Moss *et al.* 2011; Vinebrooke, Rolf *et al.* 2004).

Eutrophication is the enrichment of waterbodies with nutrients and its effects. Nutrients are essential for the growth and reproduction of aquatic plants, but when a waterbody becomes enriched with nutrients it typically leads to an increase in biological productivity and a decrease in biodiversity (Smith *et al.* 1999; Wetzel 2001). Although the process of eutrophication is naturally occurring, the urban, industrial and agricultural use and subsequent disposal of plant nutrients since the Industrial Revolution have accelerated and amplified the process in many lakes (Harper 1992). Consequently, eutrophication has been the paramount stressor on lake ecosystems since the mid-twentieth century (Maltby & Ormerod 2011; Moss *et al.* 2011; Smith 1998, 2003).

The Redfield ratio suggests that phytoplankton require carbon, nitrogen and phosphorus in a ratio of 106:16:1 for optimal growth (Redfield 1958). Where concentrations of these nutrients are below the levels required by primary producers, they place a limit on the rate of growth and reproduction (Hecky & Kilham 1988). As concentrations of phosphorus are often relatively low compared to those of other essential nutrients in lakes, it is widely considered to be the main limiting factor (Marsden 1989; Schindler *et al.* 2016). This assumption is validated by the meta-scale relationship between concentrations of phosphorus and chlorophyll *a*, a proxy for phytoplankton biomass (Vollenweider 1968). The elevated input of a limiting nutrient disrupts the natural stoichiometry of the lake and results in changes to the primary producer community.

Increased phosphorus loading to lakes with the intensification of agriculture and expansion of populations connected to wastewater treatment works (WwTWs) has created ideal conditions for enhanced phytoplankton production. Phosphorus enrichment particularly favours cyanobacteria by reducing nitrogen to phosphorus ratios, which promotes the growth of heterocystous species (i.e. those with ability to fix atmospheric nitrogen). Cyanobacteria, more so than other phytoplankton taxa, are problematic because some species produce harmful cyanotoxins, which pose a health risk to both humans and animals (Codd 2000). The development of blooms also impacts on overall ecosystem structure. Blooms can reduce light penetration, which potentially limits the growth of submerged macrophytes (Jones *et al.* 1983; Jupp & Spence 1977). Sedimentation of the readily degradable organic matter produced by the bloom stimulates

microbial mineralisation at the sediment surface, thereby increasing the probability of hypolimnetic anoxia and the release of sediment-bound phosphorus to the overlying water column (Phillips *et al.* 1994; Ripl 1986). Conditions of hypoxia or anoxia may be further exacerbated by the night-time respiration of algal blooms (Paerl *et al.* 2001). Consequently, populations of species less tolerant of reduced oxygen levels (e.g. salmonids) decline. In extreme cases, the combination of hypolimnetic hypoxia or anoxia and toxic algae has been reported to cause fish kills (Muller & Stadelmann 2004).

Dealing with the consequences of nutrient enrichment can be costly. For example, Pretty *et al*. (2003) estimated that responding to eutrophication problems in England and Wales costs US$ 105 to 160 million per year. This estimate accounts for the reduced value of waterfront dwellings, increased drinking water treatment costs for the removal of cyanotoxins and decomposition products, reduced recreational and amenity value of waterbodies and economic losses from the tourist industry, among others.

Recognition of the threat that eutrophication and other stressors pose to the sustainable use and development of vital ecosystem services has led to the introduction of legislative programmes in some regions to improve water quality and prevent further degradation. In states of the European Union, the Water Framework Directive (EU WFD) was implemented in the year 2000. It stipulates that unmodified, natural waterbodies should be returned to Good Ecological Status by the years 2015 to 2027 (European Union 2000). According to the EU WFD, this target is characterised by "low levels of distortion resulting from human activity but deviates only slightly from those normally associated with the surface waterbody type under undisturbed conditions". Pre-1850 conditions are commonly used as targets, as this was prior to the onset of the Industrial Revolution and rapid population growth (Bennion *et al.* 2011). The Directive identifies a number of approaches that can be used to determine reference conditions for lakes, including historical data, expert judgement, mathematical modelling and palaeolimnology (Wallin *et al.* 2005). In the years since the EU WFD was implemented, determining the most effective strategies for the management and remediation of eutrophic lakes has become a major focus of limnological research (Mackay *et al.* 2014a; Søndergaard *et al.* 2007, 2008; Zamparas & Zacharias 2014).

The response of lake ecosystem structure and function to eutrophication and other stressors is controlled by numerous processes, operating at different spatial and temporal scales and involving complex interactions between different trophic levels (Faithfull *et al.*

2011; Maberly & Elliott 2012; Moss 2012). Trophic interactions can either be classified as bottom-up or top-down (Hansson 1992; Power 1992). Bottom-up forces relate to the effects of resource availability upon ecosystems and constituent food webs, the most significant of which in many lakes is the link between phosphorus loading and phytoplankton biomass (Schindler 1974; Vollenweider 1968). On the other hand, top-down processes involve the regulation of biomass, community structure or productivity at a certain trophic level by predation or grazing from the level above; known as a trophic cascade (Carpenter *et al.* 1985; Jeppesen *et al.* 1997). Whether top-down or bottom-up forces have primacy in lake ecosystems has previously been the subject of much debate. It is now widely accepted that these two opposing forces make a concomitant contribution to shaping lake ecosystems, but that their relative importance varies depending on environmental conditions (Benndorf *et al.* 2002; Leibold *et al.* 1997; McQueen *et al.* 1989; Pace *et al.* 1999).

Lake managers apply the concepts of top-down and bottom-up control to the remediation of eutrophic lakes. Most remediation projects have focussed on reducing eutrophication by controlling external nutrient loads. Bottom-up regulation occurs when a change in nutrient supply leads to similar changes in equilibrium biomass at all trophic levels (Figure 1.1), at least until biomass is constrained by other factors (Kagata & Ohgushi 2006). As phosphorus is often assumed to be the main nutrient limiting phytoplankton growth, it is the focus of most eutrophic lake restoration programmes, rather than other essential growth nutrients like nitrogen or silica (Marsden 1989; Schindler *et al.* 2016). Typically, bottom-up management involves improving the efficiency of phosphorus stripping by wastewater treatment facilities (Hall *et al.* 1993; May *et al.* 2012; Søndergaard *et al.* 2007). However, a reduction in the external phosphorus load does not necessarily translate to an immediate decrease in lake water phosphorus concentration and improved ecological condition. Many case studies have highlighted a lag time of years, and in some instances decades, before water quality improvements are observed (Bailey-Watts & Kirika 1999; D'Arcy *et al.* 2006; Jeppesen *et al.* 1991; Marsden 1989; Søndergaard *et al.* 2007).

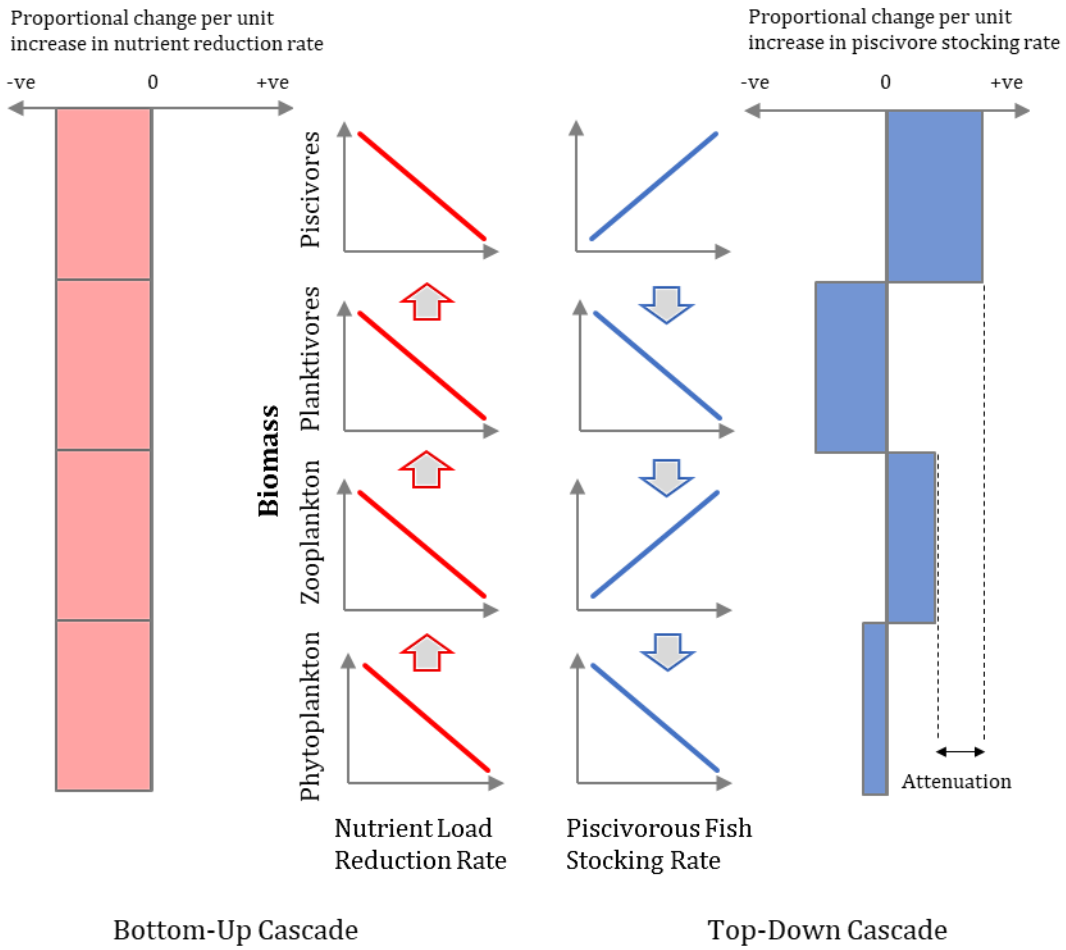

Figure 1.1: Schematic representation of bottom-up and top-down forced trophic cascades, showing the conceptual patterns of correlation and attenuation between adjacent trophic levels. Adapted from Heath *et al.* (2014).

The delayed recovery may be a chemical resistance conditioned by the release of phosphorus from a pool accumulated in the sediment during the period of high external loading. Depending on the loading history and release mechanisms, this phase typically persists for 10 to 15 years after the external loading reduction until new equilibrium conditions are established between the water column and lake sediments (Marsden 1989; Phillips *et al.* 1994; Sas 1989; Søndergaard *et al.* 2001). Secondary in-lake remediation measures (e.g. phosphorus-capping agents) are increasingly being applied following external load reductions to minimise the internal phosphorus load and accelerate the recovery trajectories of eutrophic lakes (Huser *et al.* 2016; Meis *et al.* 2013).

The modest scale of ecological responses to remediation measures may also be due to resistance caused by biological rather than chemical mechanisms. For example, the food web structure established when external loading was high. Productive lakes are often

dominated by large stocks of zooplanktivorous and benthivorous fish species, which can impede the top-down control of zooplankton on phytoplankton (Meijer *et al.* 1999; Persson *et al.* 1988; Shapiro & Wright 1984). This biological resistance has been overcome by biomanipulation (Beklioglu *et al.* 2003; Shapiro & Wright 1984; Søndergaard *et al.* 2008). A common approach involves the removal of zooplanktivorous fish, either by capture or by the introduction of piscivorous fish to reduce the abundance of zooplanktivores by predation (Jeppesen *et al.* 2012; Søndergaard *et al.* 2007). An efficient reduction in their biomass generally achieves dramatic, short-term cascading effects in eutrophic lakes, though the effect is often progressively attenuated with transfer between successive trophic levels (as shown in Figure 1.1). The desired cascading effect is for a shift towards dominance by large zooplankton, reduced phytoplankton biomass caused by intensified grazing pressure, and improved water transparency as phytoplankton biomass decreases (Carpenter & Kitchell 1993). However, the long-term effects of biomanipulation have been variable. Observations from a number of lakes suggest that the success of long-term water quality improvements following biomanipulation is greatly improved if substantial reductions in external phosphorus load are made simultaneously (Benndorf 1990; Jeppesen *et al.* 1999; Søndergaard *et al.* 1990, 2008).

Over the last few decades, global climate change has been recognised as a potential threat to water quality and lake functioning (Dossena *et al.* 2012; George 2010; Jeppesen *et al.* 2010; Shurin *et al.* 2012). Increasing water temperatures and associated changes to thermal structure, for example stronger and longer-lasting thermal stratification, are already apparent in many lakes (De Stasio *et al.* 1996; Wagner & Adrian 2011). This may lead to a change in plankton phenology and a mismatch in seasonal timing between trophic levels (Adrian *et al.* 2006; Winder & Schindler 2004a). The changes in lake thermal structure are also likely to favour toxin producing cyanobacteria over other phytoplankton groups, thus contributing to water quality deterioration (Carey *et al.* 2012; Paerl & Huisman 2008). In lakes already impacted by high nutrient inputs, global warming and eutrophication tend mutually to reinforce the symptoms they express (Kosten *et al.* 2011, 2012; Moss *et al.* 2011). These changes could disrupt established interactions between top-down and bottom-up processes, confounding the management and recovery of eutrophic lakes.

In 2015, 15 years after the implementation of the EU WFD, 47 % of EU surface waters had not reached Good Ecological Status, the central objective of EU water legislation (Voulvoulis *et al.* 2017). Clearly, the processes that control lake ecosystem structure and

function are complex. A better understanding of these processes and of how they can be effectively manipulated to remediate eutrophic lakes is vital if legislative targets are to be met and ecosystem services secured into the future. This thesis examines the relative importance of top-down and bottom-up processes in regulating lake ecosystems, with a particular focus on phytoplankton communities. Additionally, perturbations to the balance of these processes due to eutrophication and climate change are investigated. The findings are applied to determining suitable remediation strategies for eutrophic lakes in poor ecological condition.

Esthwaite Water in the English Lake District is the ideal model lake to use in these investigations. Water quality data have been collected from Esthwaite Water since the 1940s and human activities in the lake and surrounding catchment, such as sewage treatment processes and aquaculture, have been well documented (Maberly *et al.* 2011; Talling & Heaney 1983). Following several decades of eutrophication, a number of remediation measures have been implemented in an attempt to attain EU WFD Good Ecological Status for Esthwaite Water. Essentially, lake managers have unintentionally conducted an ecosystem-scale experiment in Esthwaite Water. This presents a relatively unique opportunity to disentangle the effects of anthropogenic perturbations from natural water quality variability over time.

1.2 Objectives and thesis structure

The role of top-down and bottom-up processes, and their interactions, is critical in regulating lake ecosystem structure and water quality. The impact of human activities on environmental processes at local, regional and global scales has the potential to disrupt the natural balance of these processes. In particular, there is a significant gap in our knowledge regarding the combined effects of nutrient enrichment and climate change on lake structure and function, and the consequences of these effects for the long-term management of lakes and associated ecosystem services.

The overarching objectives of this thesis were: (1) to investigate the role of top-down and bottom-up processes, and their interactions, in regulating lake ecosystem structure and function; and (2) to determine how the relative importance of these two opposing processes varies in response to anthropogenic perturbations, specifically eutrophication and climate change. It was hypothesised that eutrophication and climate change will both shift the balance of control towards bottom-up processes, and that the recovery of Esthwaite Water following remediation measures will be delayed by a combination of internal phosphorus loading and the exacerbation of eutrophic conditions due to climate change impacts.

To achieve the main objectives, several more specific aims were investigated. They were as follows:

(i) To quantify historical lake phosphorus budgets, including the flux of phosphorus across the sediment-water interface (internal phosphorus load).
(ii) To determine the historical drivers of water quality variability.
(iii) To quantify the current inventory of phosphorus in lake sediments and determine the proportion of release sensitive phosphorus.
(iv) To determine the impact of aquaculture on the content and fractionation of sediment phosphorus.
(v) To determine how the relative importance of top-down (grazing) and bottom-up (nutrient availability) forces varies seasonally, and the potential for climate change to affect the balance of these processes.
(vi) To examine possible recovery trajectories from eutrophication and investigate how internal phosphorus loading and climate warming could impact recovery.

Chapter Two introduces the study site, Esthwaite Water, and provides context for the work reported in the following data chapters. **Chapter Three** addresses objectives (i) and (ii). Annual lake phosphorus budgets were compiled from long-term monitoring data and data from previous short-term studies for each year between 1970 and 2014. Long-term water quality monitoring data, meteorological data and fish stocking data were analysed to determine the historical drivers of water quality change. **Chapter Four** reports on the analyses of contemporary lake sediments to determine the spatial variability of phosphorus content and fractionation in relation to the former location of salmonid culture cages [objectives (iii) and (iv)]. The lake model PROTECH was utilised in **Chapter Five** to address objectives (v) and (vi). Year-long simulations of lake phytoplankton community were conducted under different scenarios of nutrient loading, zooplankton grazing pressure and climate warming to assess the individual and combined effects of these determinants. Long-term simulations were also run to investigate the impact of climate change and internal loading on the recovery of eutrophic lakes. **Chapter Six** presents a discussion of the work as a whole, highlights implications of the findings for lake management and identifies areas for further research.

Chapter Two

Study Site: Esthwaite Water

2.1 Basic features

Esthwaite Water (54° 21' N, 2° 59' W) is a small lake of glacial origin in the south-eastern area of the English Lake District, Cumbria, UK. It has a small drainage area of 17.1 km^2 which forms part of the larger Windermere catchment (Figure 2.1). Esthwaite Water covers an area of 0.96 km^2 and has a total volume of $6.7x10^6$ m^3 (Mackay *et al.* 2012). According to EU Water Framework Directive (WFD) typology it is categorised as a shallow lake, based on its mean depth of 6.9 m (European Union 2000). The lake is separated by sills into three main basins, of which the northernmost basin is the largest (0.54 km^2) and deepest (maximum depth 16 m). Black Beck, entering at the northern shallow bay, is the main inflow accounting for *c*. 55 % of the hydraulic load to the lake (Maberly *et al.* 2011). Five smaller tributaries also discharge into the lake. The sole outflow, Cunsey Beck, exits to the south and discharges after 3.2 km into the south basin of Windermere. The average discharge of 0.93 m^3 s^{-1} recorded between 1976 and 2014 gives a mean retention time of *c*. 83 days. The lake is designated as a Site of Special Scientific Interest by Natural England on account of its diverse macrophyte community and was also designated as a Ramsar site in 1997 in recognition of its international importance as a wetland.

2.2 Catchment geology and land cover

Esthwaite Water and its catchment lie almost entirely on Lower Palaeozoic, Silurian rock (formed 450 to 400 million years ago), known as the Bannisdale Slates (Fryer 1991). The calcareous nature of the clayey siltstone and mudstone from which these slates are comprised accounts for the high alkalinity of Esthwaite Water compared to other lakes in the region (Reynolds & Irish 2000; Sutcliffe 2010; Sutcliffe & Carrick 1983). The EU WFD categorises Esthwaite Water as a medium alkalinity lake. Of the 20 Cumbrian lakes included in the most recent Lakes Tour (refer to Section 2.4 for details), Esthwaite Water had the highest mean alkalinity at 440 µEq l^{-1}, compared to an overall mean of 197 µEq l^{-1} (Maberly *et al.* 2016). A thin band of Coniston Limestone (deposited 350 to 270 million years ago) runs through the very north of the catchment area (Moseley 1978). Glacial till overlies the bedrock geology on the lower slopes of the valley, while alluvial and lacustrine deposits surround the lake itself (British Geological Survey 1998). Catchment soils are predominantly free-draining brown earths composed of silt loam and sandy loam with poorly-drained peaty gley soils in places (Hall & Folland 1970). The catchment once supported deciduous woodlands comprising, oak (*Quercus petraea*), ash (*Fraxinus excelsior*) and yew (*Taxus baccata*). However, much of the former forest was cleared to make way for pastoral agriculture and commercial forestry (Reynolds & Irish 2000), which comprise 45 % and 41 % of the catchment area, respectively (May *et al.* 1997).

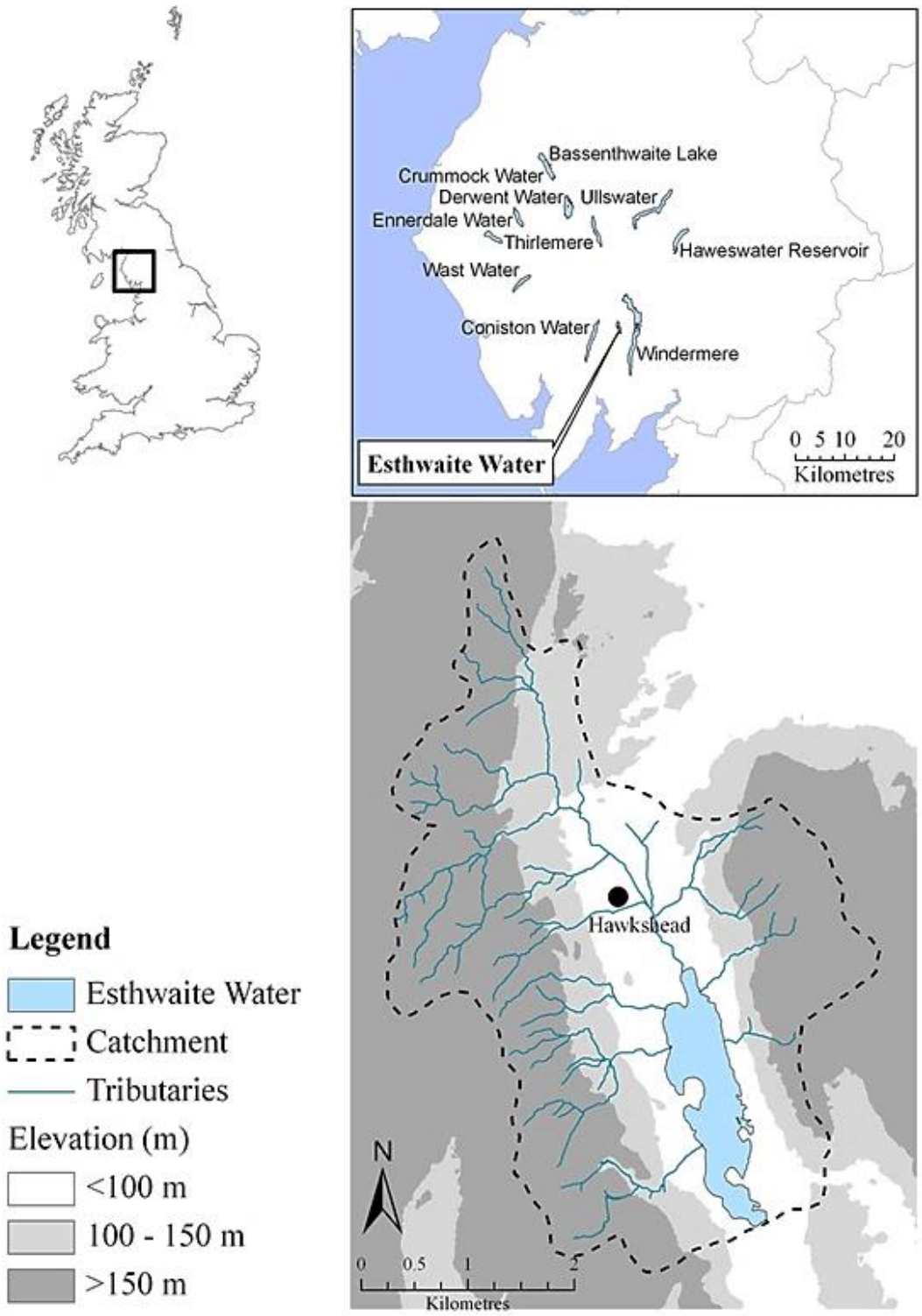

Figure 2.1: Location of Esthwaite Water and its catchment area in the UK. Source: Mackay (2011).

The village of Hawkshead lies within the catchment area to the north of the lake. The relatively small resident population (519; Office for National Statistics 2011) increases considerably with seasonal tourism.

2.3 Climate background

The proximity of the Lake District to the western seaboard of the country strongly influences the region's climate. Annual mean rainfall is high due to westerly winds from the Atlantic Ocean forcing moist air over the Cumbrian hills where it cools, and the moisture condenses to form rain. The spatial distribution of rainfall over the Lake District is extremely heterogeneous owing to its mountainous topography and associated orographic rainfall processes (Barker *et al.* 2004; Jones & Conway 1997); annual totals range from 0.92 m y^{-1} at Kendal (Tufnell 1997) to over 3.00 m y^{-1} at Seathwaite (Manley 1946). Meteorological data has been collected at Ambleside (8 km northwest of Esthwaite Water) since 1961. The average annual rainfall (1965 to 2014) at this site, taken as an approximation of rainfall over the Esthwaite Water catchment, is relatively high at 2.00 m y^{-1}. The catchment generally experiences a rainy season between September and February, with precipitation decreasing in the middle of the year. There is also considerable variation in air temperature throughout the year. This seasonal variation results in the thermal stratification of Esthwaite Water, typically from mid-May through to the end of September, with the hypolimnion becoming anoxic during this period (George & Hewitt 1999). Figure 2.2 illustrates average seasonal weather patterns.

Esthwaite Water has been shown to respond to several regional weather patterns. In terms of inter-annual variability, the climatic conditions of the region are sensitive to fluctuations in the sea-level atmospheric pressure gradient between the Azores High and Icelandic Low, known as the North Atlantic Oscillation [NAO (Walker & Bliss 1932)]. Winter weather conditions in particular are strongly influenced by the NAO. During the positive phase of the NAO, westerly airflows are enhanced due to a large pressure difference, which leads to mild, wet and windy winters. Conversely, when the pressure gradient is reduced during the negative phase, westerlies are suppressed and a colder, drier climate prevails (Hurrell 1995; Hurrell *et al.* 2003). Analysis of long-term monitoring data has shown that the NAO is one of the most important factors affecting physical and chemical characteristics of Esthwaite Water. George *et al*. (2004) found that winter water temperatures and nutrient concentrations were highly correlated to the NAO index. Certain biological components of the lake ecosystem, particularly phytoplankton and zooplankton, also respond to NAO-related variability in climatic conditions (George 2000; George *et al.* 2004; George & Hewitt 1999).

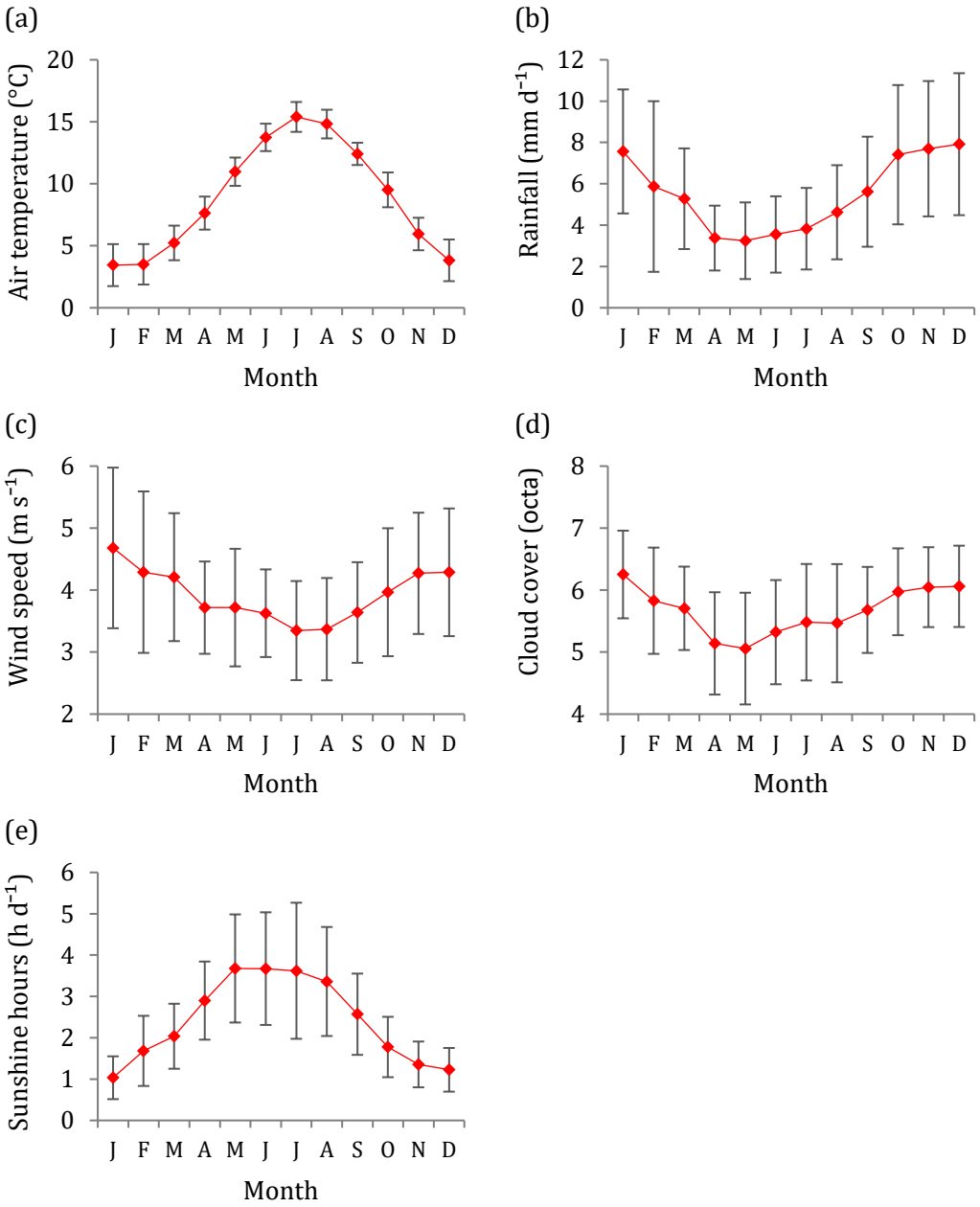

Figure 2.2: Average monthly weather patterns for the period 1965 to 2014 at Laurel Bank, Ambleside: a) air temperature, b) rainfall, c) wind speed, d) cloud cover, and e) sunshine hours. Error bars show the standard deviation of monthly means.

The influence of the NAO on lake conditions is diminished during the summer. Instead, meteorological variability associated with latitudinal displacement of the Gulf Stream in the western Atlantic has been shown to have a significant effect on the timing and intensity of thermal stratification, which in turn influences summer phytoplankton and zooplankton biomass (George 2000, 2002; George & Taylor 1995). Recent work by Strong & Maberly (2011) also highlights the significance of the large-scale atmospheric phenomenon known as tropospheric Rossby wave breaking (RWB) in controlling the

surface temperature of Cumbrian lakes, including Esthwaite Water. RWB occurs frequently near the Lake District, bringing warm and moist air, or cool and dry air from distant climes. Because this effect operates in all seasons it is potentially more important than the NAO as a driver of year-to-year variation in a wide range of lake properties.

2.4 Long-term monitoring programme

Esthwaite Water is one of the most intensively studied lakes in the world. The Freshwater Biological Association (FBA) initiated a long-term monitoring programme in 1945, subsequently undertaken by the Institute of Freshwater Ecology and Centre for Ecology & Hydrology (CEH) since 1989. A wide range of physical, chemical and biological variables are recorded at the lake on a fortnightly basis. More recently, CEH has installed an Automatic Water Quality Monitoring Station in the lake and a meteorological station on the shore, which collect high-resolution meteorological and hydrographic data to contribute to the UK Lake Ecology Observatory Network (Rouen *et al.* 2005; Woolway *et al.* 2015). The data that result from the two monitoring schemes provide an invaluable resource for advancing our understanding of how lake ecosystems respond to environmental perturbation. Seminal work conducted early in the monitoring programme has been summarised by Talling & Heaney (1983) and Maberly *et al*. (2011). Contemporary investigations span a broad range of topics, including: phytoplankton phenology (Feuchtmayr *et al.* 2012), long-term and seasonal zooplankton dynamics (George 2012; George *et al.* 1990; George & Hewitt 1999), physical processes (Mackay *et al.* 2011; Woolway *et al.* 2014) and algal community modelling under different climate change scenarios (Elliott 2010).

Esthwaite Water is also included in the Lakes Tour, a survey of the 20 major lakes and tarns of the English Lake District that has been conducted approximately every five years since 1984. A range of water chemistry and ecological variables are surveyed seasonally at each site over one year. Data from the Lakes Tour has been used to determine ecological quality boundaries for the implementation of the EU WFD. It also serves to identify lakes that may be showing signs of deterioration and which deserve further study, as well as documenting the recovery of lakes that have already been subject to management (Maberly *et al.* 2016).

2.5 Bottom-up stressors: History of eutrophication

Esthwaite Water has been ranked several times as the most productive of the major Cumbrian lakes (Gorham *et al.* 1974; Jones 1972; Pearsall 1921). It is naturally mesotrophic (i.e. intermediate nutrient levels) owing to relatively high nutrient export from the catchment soils, but a long history of human activity in the region has elevated the nutrient load to the lake above naturally occurring levels (May *et al.* 1997; Pearsall 1921). Palaeolimnological records reveal little evidence of changing nutrient status between *c*. 800 and 1850 AD, followed by a gradual and accelerating increase between 1850 and the early 1970s (Bennion *et al.* 2000; Dong *et al.* 2011). A more dramatic increase is evident thereafter, both in the palaeolimnological record (Dong *et al.* 2012) and in the long-term monitoring data (George *et al.* 1990; Talling & Heaney 1983).

The earlier phase of eutrophication has been attributed to the increased use of artificial fertilisers in the catchment area, the advent of phosphorus-based detergents, commercial forestry operations and the growth of tourism in the region following the establishment of a railway connection to Windermere in 1847 (Bennion *et al.* 2000; McGowan *et al.* 2012; Rollinson 1967). The onset of the latter phase of eutrophication is coincident with the founding of a wastewater treatment works (WwTW) to serve the village of Hawkshead which discharges treated effluent to the main inflow (Agar *et al.* 1988; Talling & Heaney 1988). A further external nutrient source was introduced in 1981 with the installation of a fish farm for rainbow trout (*Oncorhynchus mykiss*) cultivation in the southern basin of the lake, and the consequent input of food and waste in various forms emanating from aquaculture cages to the water column (Hall 1991; Hall *et al.* 1993).

The enrichment of Esthwaite Water is best illustrated by the long-term trend in winter soluble reactive phosphorus (SRP) concentrations (Figure 2.3). At this time of year, SRP is consistently high as there is relatively little assimilation by phytoplankton and maximal replenishment by inflows (Sutcliffe *et al.* 1982). Prior to the establishment of Hawkshead WwTW in 1973, the annual winter mean SRP concentration rarely exceeded 3 μg l^{-1}. Thereafter, the concentration gradually increased to a maximum of 18.4 μg l^{-1} in 2001. Although steps were taken by United Utilities, and their predecessors North West Water, in the 1980s and 1990s to reduce the external nutrient load by upgrades to wastewater treatment facilities, nutrients derived from catchment run-off and the fish farm appear to have negated any potential reduction in lake nutrient concentrations. It has also been speculated that phosphorus release from lake sediments has played a role in maintaining

the water column concentrations (Hall *et al.* 1993; Mackay *et al.* 2012; Talling & Heaney 1983).

Nutrient enrichment has had a profound effect on the ecology of Esthwaite Water. The most notable symptoms have been an increase in the overall abundance of phytoplankton concurrent with the period of water quality deterioration (Maberly *et al.* 2011), and prolonged occurrence of cyanobacterial blooms throughout summer and autumn (Heaney *et al.* 1992). It is evident in the sediment fossil record that the diatom community composition has shifted away from species characteristic of low nutrient conditions (e.g. *Cyclotella comensis*, *Cyclotella radiosa* and *Achnanthidium minutissimum*) towards those with a higher tolerance for eutrophic environments [e.g. *Asterionella formosa* and *Fragilaria crotonensis* (Bennion *et al.* 2000; Dong *et al.* 2011)]. The once diverse macrophyte community has also been impacted. An early study by Pearsall (1917) recorded 22 species of submerged or floating macrophytes in Esthwaite Water, including the nationally rare *Najas flexilis*. Subsequent surveys have recorded a 50 % loss in macrophyte diversity and *Najas flexilis* now appears to be extinct in Esthwaite Water (Darwell 2000).

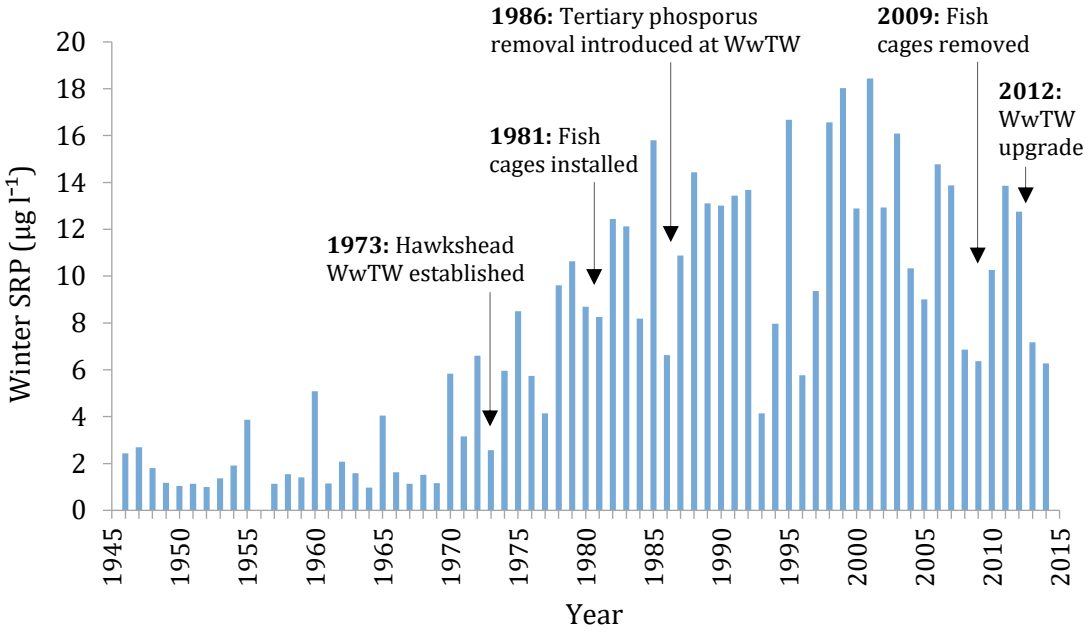

Figure 2.3: Annual winter SRP concentration (mean of December, January and February measurements), Esthwaite Water, 1946 to 2014. Data collected on a fortnightly basis as part of the CEH long-term monitoring programme.

2.6 Top-down stressors: The introduction of farmed trout

In 1983, two years after the installation of aquaculture cages, the fishery began to release a component of annual production to the lake for recreational fishing (*c*. 11,000 fish released annually). The introduction of this non-native species may have triggered a trophic cascade by increasing the predation pressure on macroinvertebrates and smaller fish species, and in turn reducing the predation pressure on zooplankton. The zooplankton may have been further relieved from predation due to the supplementary feeding of native planktivorous fish on uneaten food waste from the fish culture cages. Grey *et al*. (2004) used stable isotope analysis to investigate the fate of waste pelleted food in Esthwaite Water. They concluded that roach (*Rutilus rutilus*) were probably short-circuiting the lake's food chain by directly consuming significant quantities of particulate pellet material, to the extent that 80 % of their body carbon may ultimately have been derived from fish food.

Unfortunately, the fish population of Esthwaite Water has been subject to relatively little investigation. Limited information is available regarding changes to species composition and abundance since the long-term monitoring programme began. A brief description of the fish population in 1989 noted that brown trout (*Salmo trutta*), pike (*Esox lucius*) and perch (*Perca fluviatilis*) were the main species, with roach and rudd (*Scardinius erythrophthalmus*) also present (Frost 1989). The most recent survey, conducted in August 2013, determined that the fish population is currently dominated by roach and perch, a scenario typical of eutrophic lakes (Winfield *et al.* 2014). The native brown trout population appears to have become severely reduced or to have died-out altogether, perhaps as a result of competition for resources with the larger, more aggressive stocked trout or predation of juvenile brown trout by the stocked trout (Welton *et al.* 1997).

2.7 Recent remediation efforts

In an attempt to reach ecological targets by 2027, Natural England bought-out the fish farm in 2009 and removed the trout culture cages from the lake. Since 2011, the number of fish released for recreational fishing has been reduced to around 7,500 (D. Coleman of Esthwaite Water Trout Fishery, pers. comm., 2015) and, in accordance with the National Trout and Grayling Strategy (Environment Agency 2003), rainbow trout stocking was phased out in favour of brown trout. Rainbow trout appear to have been rapidly expatriated as no individuals of the species were recorded during the 2013 fish survey (Winfield *et al.* 2014). Although fewer fish are now being released the density of large predators in the lake remains above naturally occurring levels and is likely to have a

continued impact on food web dynamics. Moreover, the stocked fish may continue to act as an external nutrient source having been fed on highly nutritious pelletised food up until the point of their release.

The most recent efforts to remediate Esthwaite Water have been undertaken by United Utilities. Between 2010 and 2012 wastewater handling facilities at Hawkshead were upgraded extensively. Table 2.1 details the timeline of lake remediation efforts and other relevant human activities in the catchment area since long-term water quality monitoring began in 1946. The impact of these changes on lake water quality is investigated in Chapter Three.

Long-term water quality monitoring data suggests some improvement in recent years. As shown in Figure 2.3, annual winter mean SRP concentration decreased from 18.4 μg l^{-1} in 2001 to 6.3 μg l^{-1} by 2014. However, Esthwaite Water is not currently of Good Ecological Status as required by the EU WFD. The 2015 Lakes Tour classified it at Moderate Ecological Status, based on the annual mean concentration of total phosphorus (TP, 17.5 μg l^{-1}) and the annual mean modelled chlorophyll *a* concentration (16.1 μg l^{-1}). The site-specific EU WFD boundaries for achieving Good Ecological Status are 16.4 μg l^{-1} for TP and 9.6 μg l^{-1} for chlorophyll *a* (Maberly *et al.* 2016).

Table 2.1: Timeline of lake remediation efforts and other significant anthropogenic activities in Esthwaite Water and its catchment area since the start of long-term monitoring in 1946.

Year	Activity	Details
1973	WwTW established to serve the village of Hawkshead.	- Treated effluent discharged into Black Beck, the main inflow to Esthwaite Water.
1981	Esthwaite Water Trout Fishery installed fish culture cages in the south basin of the lake.	- Approximately 200,000 rainbow trout (150 tonnes) produced annually in floating cages.
1983	The fishery began stocking the lake with rainbow trout for recreational fishing.	- Approximately 11,000 fish (8.5 tonnes) released annually.
1986	Tertiary phosphorus removal implemented at Hawkshead WwTW.	- Phosphorus stripping by chemical precipitation. - Initially carried-out between April and October then year-round from 1989.
1993	Redirection of Near Sawrey WwTW effluent outflow.	- Originally discharged directly into Esthwaite Water. - Redirected to the outflow, Cunsey Beck.
1998	Switch to organic farming by the fishery.	- Annual rainbow trout production reduced to approximately 67,000 fish (50 tonnes) per year.
2006	Catchment Sensitive Farming scheme implemented in England.	- Joint initiative of the Environment Agency, Natural England and Defra. - Promotes voluntary action by farmers in priority catchments to tackle the problems of diffuse water pollution from agriculture. - Esthwaite lies within the Rivers Kent and Leven Catchment.
2009	Fish culture cages removed from Esthwaite Water.	- Fish farm activities bought-out by Natural England.
2010 – 2012	Hawkshead WwTW underwent major upgrades.	- Upgrade of tertiary treatment process to reduce the phosphorus concentration of the final effluent. - System capacity increased to reduce the occurrence of intermittent discharges of untreated effluent.
2011 – 2013	Fishery phased out stocking of rainbow trout in favour of brown trout.	- Stocking reduced to approximately 7,500 trout (4.5 tonnes) per year.

Chapter Three

Long-term changes in phytoplankton indicators of lake water quality in response to multiple anthropogenic stressors

In this chapter, long-term environmental monitoring data were analysed to determine the impact of catchment-based and in-lake anthropogenic activities on the water quality of Esthwaite Water, against a background of ongoing climate change.

3.1 Introduction

Humankind relies on lakes for the provision of numerous essential goods and resources. These include, but are not limited to, the supply of water for domestic, industrial and agricultural purposes, food production, waste disposal and recreation (Maltby & Ormerod 2011; Wetzel 2001). Lakes face a variety of pressures arising from catchment-based anthropogenic activities and long-term changes in background environmental conditions, which greatly threaten their ecological integrity (Allan *et al.* 2013; Dudgeon *et al.* 2006; Vörösmarty *et al.* 2000). Consequently, lake degradation has become a major cause for concern throughout the world and increasing efforts are being made to protect lakes and to secure the vital ecosystem services that they provide (Carpenter & Cottingham 1997; Cooke *et al.* 2005; Søndergaard *et al.* 2007). In Europe, focus on this issue has intensified since 2000 with the implementation of the EU Water Framework Directive (WFD), which stipulates that natural waterbodies must be returned to Good Ecological Status by 2027 (European Union 2000).

The availability of nutrients, particularly phosphorus, has long been recognised as a key factor in determining lake ecological condition (Correll 1998; Schindler 1977; Vollenweider 1968). Phosphorus enrichment of lakes from point and diffuse sources of agricultural, sewage and industrial wastes within the catchment is common in densely populated regions (Bennett *et al.* 2001; Carpenter *et al.* 1998; Hall *et al.* 1999; McGowan *et al.* 2012). The resultant increase in nutrient availability in the lake typically promotes phytoplankton growth, including toxin-producing cyanobacteria, which in turn has significant negative impacts on water quality, biodiversity and functioning of the lake (Harper 1992; Smith *et al.* 1999). Traditionally, lake restoration efforts have focused on reducing the phosphorus load from catchment sources to lakes. Although some lakes respond rapidly to changes in the external phosphorus load (Sas 1989), water quality improvements have frequently been slow, commonly taking many years to decades for the recovery process to end (D'Arcy *et al.* 2006; Jeppesen *et al.* 1991; Marsden 1989). The reason for this delayed recovery is that phosphorus accumulated in sediments during periods of high external loading cycles between water column and bed sediments, which acts to slow relinquishment from the lake (Søndergaard *et al.* 2001). Although this internal load diminishes over time, it may be intense enough to maintain high lake water phosphorus concentrations and subsequently poor ecological conditions for many years following external load reductions (Jeppesen *et al.* 2005; Søndergaard *et al.* 2003; Spears *et al.* 2012).

Lakes that are afflicted by eutrophication are also often subjected to other local and widespread anthropogenic pressures, which further complicates their management (Christensen *et al.* 2006; Maberly & Elliott 2012; Smol 2010). At the local scale, the introduction of non-native species is one such pressure. Fish are often introduced to freshwaters, either by accident or deliberately to provide food or sport (Strayer 2010). Nutrient availability may determine potential phytoplankton biomass although biomass accrual can be limited through grazing by zooplankton (Carpenter *et al.* 1985). Zooplankton grazing is in turn influenced by the abundance of zooplanktivorous fish (Brooks & Dodson 1965; Hrbácek *et al.* 1961). Therefore, fish stocking and the associated change in predation pressure can cascade through trophic levels to zooplankton and thus alter the grazing pressure exerted on phytoplankton. Whether the impact on phytoplankton is positive or negative depends on the species of fish introduced to the lake and pre-existing food web structure (Matsuzaki *et al.* 2009).

In recent decades, global climate change has emerged as a serious threat to the structure and function of lake ecosystems (George 2010; Jeppesen *et al.* 2010; Shurin *et al.* 2012). The most studied effects of climate change on lakes have been those related to warming and its impact on phytoplankton. Phytoplankton community data from lake monitoring (Jöhnk *et al.* 2008; Kosten *et al.* 2012; Posch *et al.* 2012), experimental mesocosms (Rasconi *et al.* 2017) and modelling (Elliott 2010) indicate that the abundance of cyanobacteria relative to other phytoplankton groups increases with increasing temperature. Cyanobacteria have a higher optimum growth temperature than many other phytoplankton groups, and elevated water temperatures can favour cyanobacteria directly (Butterwick *et al.* 2004). Additionally, changes in lake physical properties connected to increasing air temperature indirectly benefit cyanobacteria. Some species have ecophysiological adaptations, such as buoyancy regulation (e.g. *Microcystis*) and the ability to fixate atmospheric nitrogen (e.g. *Dolichospermum*), which give them a competitive advantage over other species in nutrient-depleted, poorly-mixed water column conditions (Carey *et al.* 2012; Paerl & Huisman 2008). Climate warming causes lakes to stratify earlier in the spring and turnover later in the autumn (De Stasio *et al.* 1996), thereby lengthening the annual period of optimal growth conditions for cyanobacteria (Wagner & Adrian 2009, 2011).

The effect of global warming on precipitation patterns could further enhance cyanobacterial dominance. Often associated with increasing temperatures are an overall decrease in precipitation during the summer and an increase in the frequency of extreme

precipitation events (IPCC 2014b). A decrease in overall summertime rainfall may contribute to favourable conditions for cyanobacteria growth due to potentially longer periods of high evaporation and stratification (Reichwaldt & Ghadouani 2012). On the other hand, short-term periods of heavy rainfall could temporarily inhibit the development of cyanobacteria blooms due to flushing and de-stratification (Reynolds & Lund 1988). However, heavy rainfall events are also likely to increase diffuse nutrient loading from the catchment to the lake (Heathwaite & Dils 2000). When river discharge subsides and lake residence time increases, the nutrients delivered to the lake will be captured and can trigger the development of cyanobacterial blooms (Paerl & Huisman 2008). There is growing concern that the recovery of eutrophic lakes following nutrient load reduction will be overridden by the effects of climate change on phytoplankton communities (Jeppesen *et al.* 2005; Moss *et al.* 2011).

High-resolution physico-chemical, biological and climate data from long-term monitoring programmes are an essential resource for developing our understanding of how ecosystems respond to anthropogenic perturbations (Elliott 1990; Franklin 1989; Lindenmayer & Likens 2010). This knowledge is essential for the successful management and restoration of lakes. Unfortunately, continuous lake monitoring schemes are often initiated only after water quality has deteriorated, making it difficult to determine the specific causes and mechanisms of ecosystem change. Comprehensive long-term data sets do, however, exist for a small number of well-studied sites in the UK, including Esthwaite Water in the English Lake District, where records now span over 70 years (Maberly & Elliott 2012). Additionally, human activities in the lake and catchment area, which have the potential to impact on water quality, have been reasonably well documented over the last century. Consequently, Esthwaite Water is an ideal study site with which to examine the timescales, processes and end points of responses to changes in multiple stressors.

Several events in or around Esthwaite Water in recent decades have been identified as potential drivers of significant water quality change. These events include the establishment of Hawkshead WwTW in 1973 and subsequent upgrades to the sewage treatment facilities in 1986 and again between 2010 and 2012, which resulted in changes to the external phosphorus load (Agar *et al.* 1988; Hall *et al.* 1993). The installation of salmonid culture cages and the introduction of farmed rainbow trout to the lake for recreational fishing purposes in 1981 were also considered likely to impact on phytoplankton biomass and community composition, not only by introducing a new source of nutrients but also via perturbations to natural trophic interactions.

In this study, phytoplankton biomass and community composition, specifically the relative abundance of cyanobacteria, were used as biological indicators of water quality change in Esthwaite Water. The main objectives were:

(i) To compile a long-term record of annual external phosphorus loads to Esthwaite Water, which could be used in combination with water column phosphorus concentrations to predict historical annual net sediment phosphorus fluxes by mass balance modelling.

(ii) To investigate variation in long-term water quality indicators in relation to the timing of major anthropogenic activities in the lake and catchment area, for example the establishment of Hawkshead WwTW and installation of aquaculture cages.

(iii) To determine the relationships between water quality indicators and a range of potential drivers of variability, including phosphorus availability, food web structure and climatic variables.

It was hypothesised that:

(i) Water quality would deteriorate rapidly following an increase in external phosphorus load, associated with various anthropogenic activities in the catchment area and lake.

(ii) The impact of measures to reduce the external phosphorus load implemented after water quality deterioration would be obscured by the commencement of internal phosphorus loading and the effects of ongoing and accelerating climate change on lake phytoplankton communities.

3.2 Methods

3.2.1 Data collection

Much of the data used in this study originated from an ongoing long-term monitoring programme in the pelagic zone of Esthwaite Water, UK (for study site details refer to Chapter Two). Although the monitoring scheme started in 1945, analysis here focused on the period 1970 to 2014, during which consistent methods were used to collect the data of interest at weekly to biweekly intervals. Integrated surface water samples for the determination of key biological and chemical properties were collected by lowering a weighted plastic tube to a depth of 5 m (Lund 1949). Concentrations of total phosphorus (TP, μg l^{-1}) were measured colourimetrically following the digestion of particulate phosphorus forms by exposure to persulfate-acid, according to the method of Eisenreich *et al*. (1975). Samples for chlorophyll *a* analysis were prepared by filtering a known

volume of the integrated surface water sample through a Whatman GF/C filter (pore size *c*. 1.2 μm). Following the extraction of pigments from the filter paper in boiling methanol, chlorophyll *a* concentrations were determined spectrophotometrically (Talling 1974). Water samples for phytoplankton enumeration were preserved with Lugol's iodine upon collection. Preserved samples were mixed thoroughly, and sub-samples of a known volume were sedimented in graduated measuring cylinders. The supernatant was siphoned off before the constituent phytoplankton species in the remaining sample were identified and counted by microscopic examination (Lund *et al.* 1958). Aggregate crustacean zooplankton data were derived from counts of cladocera and copepoda retained on the filter papers used in the determination of chlorophyll *a* concentration (Talling 2003). Although this method produces data of lower taxonomic resolution than the microscopic examination of zooplankton samples collected by net-hauls, population estimates obtained by the two methods during periods of overlapping use at Esthwaite Water are strongly correlated (George 2012). As the filter paper method provides the only continuous record of zooplankton abundance between 1970 and 2014, it was considered the most suitable source of data for this study.

The main inflow to Esthwaite Water, Black Beck, is sampled as part of the long-term monitoring programme. Black Beck TP concentration data were available at weekly to biweekly resolution from April 1985 onwards. Water samples were collected approximately 0.5 km upstream of the point where treated sewage effluent is discharged to Black Beck from Hawkshead WwTW. Throughout 2013 and 2014, additional monthly samples were collected for TP analysis from Black Beck and from the five smaller inflows to Esthwaite Water. Measurements of flow velocity (m s^{-1}) were taken at the same time using a current meter (C2 Current Meter, OTT Hydromet GmbH, Kempten, Germany) and multiplied by stream cross-sectional area to obtain discharge (m^3 s^{-1}). Samples of final treated effluent from Hawkshead WwTW were provided fortnightly by United Utilities throughout 2013 and 2014, which were analysed for their TP concentration. United Utilities also provided daily mean data of final effluent discharge from the WwTW for 2013 and 2014. Daily mean discharge data for the outflow from Esthwaite Water, Cunsey Beck, were available from the National River Flow Archive (NRFA) from 1976 onwards. Meteorological data were provided by a citizen scientist based at Ambleside, 8 km north of Esthwaite Water. Rainfall (mm d^{-1}), wind speed (m s^{-1}) and air temperature (°C) have all been measured at twice daily intervals throughout the study period. Esthwaite Water Trout Fishery provided a record of fish food input (kg $month^{-1}$) to aquaculture cages in the lake from June 1993 until the closure of the fish farm in 2009.

3.2.2 Quantification of external phosphorus loads

The major external phosphorus sources to Esthwaite Water during the study period were identified as diffuse run-off from the catchment area, direct rainfall over the lake, waste from in-lake salmonid culture cages, and treated sewage effluent discharged to lake inflows from local WwTWs (Hall *et al.* 1993; Maberly *et al.* 2011; Talling & Heaney 1983). The phosphorus load from each of these sources was calculated at monthly intervals from January 1970 to December 2014. The methods used for estimating external phosphorus load are outlined in this section. A detailed description of the data used to calculate the loads is provided in the Appendix.

Sufficient data were available for most of the study period (April 1985 to December 2014) to calculate the catchment phosphorus load transported to Esthwaite Water via the main inflow, Black Beck, as follows:

$$\text{Black Beck Load} = K\,(TP_{\text{BB}} \times Q_{\text{BB}}) \qquad (3.1)$$

where, K is a factor to convert from per second load to a monthly total load, TP_{BB} is the monthly mean TP concentration of Black Beck (µg l^{-1}), and Q is monthly mean discharge (l s^{-1}). Unfortunately, the five minor inflows to Esthwaite Water have not been monitored with the same regularity as Black Beck. However, previous short-term studies have estimated that the minor inflows account for *c.* 14 % of the total inflow phosphorus load (Hall *et al.* 1993; Talling & Heaney 1983). In the absence of comprehensive TP concentration and discharge data for the minor inflows, calculation of the total catchment phosphorus load (kg $month^{-1}$) was based on this estimate of catchment load apportionment between inflows:

$$\text{Total Catchment Load} = 1.14 \times \text{Black Beck Load} \qquad (3.2)$$

Prior to April 1985 there was no routine monitoring of any of the inflows. Talling & Heaney (1983) reported estimates of monthly inflow phosphorus loads made throughout 1968 and 1969. Linear interpolation between these data and the loads calculated using long-term monitoring data from 1985 onwards was used to fill the gap in the catchment phosphorus load record (refer to Appendix).

Hawkshead WwTW discharges treated sewage effluent to Black Beck. Consequently, the phosphorus load from Black Beck to Esthwaite Water is made-up of both catchment and sewage derived sources. However, the TP concentration data used in calculations to estimate catchment load were measured in samples collected upstream of the effluent

discharge point. Loads from the two sources could therefore be calculated independently. The phosphorus load from Hawkshead WwTW throughout 2013 and 2014 was calculated at monthly intervals using the same equation applied to Black Beck phosphorus load:

$$\text{WwTW Load} = K\ (TP_{\text{WwTW}} \times Q_{\text{WwTW}}) \quad (3.3)$$

where, TP_{WwTW} is the monthly mean TP concentration of discharged effluent calculated from fortnightly measures, and Q_{WwTW} is the monthly mean effluent discharge calculated from the daily mean data provided by United Utilities. Although effluent TP concentration was not measured routinely before 2013, estimates of annual phosphorus load from Hawkshead WwTW were available in reports of short-term investigations conducted at various points between 1973 and 2012 (Agar *et al.* 1988; Hall *et al.* 1993; Maberly *et al.* 2011). An annual estimate of the phosphorus load from Near Sawrey WwTW prior to effluent diversion away from the lake in 1994 was also available (Agar *et al.* 1988). The timings of these estimates were sufficient to determine mean annual WwTW phosphorus loads for each of the management regimes outlined in Table 3.1. These annual loads were distributed between months based on the monthly apportionment of Hawkshead WwTW phosphorus load in 2013 and 2014.

Hall (1991) conducted a short-term experiment in Esthwaite Water to determine the phosphorus budget of rainbow trout culture cages. Of the phosphorus added to the cages in fish food, only 37 % was retained in trout biomass. The remaining 63 % was lost from the cages to the surrounding water column as waste. It was assumed that trout phosphorus retention was consistent throughout the study period and the results of Hall (1991) were used in the quantification of monthly fish farm phosphorus loads between 1993 and 2009 as follows:

$$\text{Fish Farm Load} = 0.63\ (FF \times TP_{\text{FF}}) \quad (3.4)$$

where, FF is the mass of fish food added to the cages (kg) and TP_{FF} is the proportional phosphorus content of fish food, which varies between products (typically between 0.5 and 1 %). Fish food use was not recorded during the first 12 years of fish farm operations. For this period, the average monthly loads between 1993 and 1998 were used.

The phosphorus load introduced to Esthwaite Water by rainfall was calculated by multiplying rain water TP concentration (TP_{rain}) by the volume of rain to fall directly over the lake (V_{rain}, l):

a. $$V_{\text{rain}} = 1000\ (Dep_{\text{rain}} \times A_{\text{lake}}) \quad (3.5)$$

b. $$\text{Rainfall Load} = TP_{\text{rain}} \times V_{\text{rain}} \quad (3.6)$$

where, Dep_{rain} is rainfall depth during the month of interest (m) and A_{lake} is the surface area of Esthwaite Water (m^2), multiplication by 1000 to convert units of volume from m^3 to l. Based on the findings of a nutrient budgeting exercise at a nearby lake, TP_{rain} was set at 25 µg l^{-1} (May *et al.* 1997).

The monthly loads from each of the external phosphorus sources were summed to obtain a long-term record of annual external load. Annual loads were then averaged for each of the seven management regimes (Table 3.1) to assess how relative contributions of phosphorus changed with significant management changes either in the catchment area or in the lake itself.

Table 3.1: Timeline of significant anthropogenic changes and lake remediation efforts in Esthwaite Water and the surrounding catchment area since 1970. Changes at the WwTWs or Esthwaite Water Trout Fishery are indicated in bold underlined font.

Regime	Period	WwTW	Aquaculture	Stocking
1	1970 – 1972	Near Sawrey	No	No
2	1973 – 1980	Near Sawrey + **Hawkshead**	No	No
3	1981 – 1986	Near Sawrey + Hawkshead	**Yes**	**Yes**
4	1987 – 1992	Near Sawrey + **Hawkshead (upgraded)**	Yes	Yes
5	1993 – 2009	Hawkshead **(Nr. Sawrey effluent diverted from Esthwaite Water)**	Yes	Yes
6	2010 – 2012	Hawkshead	**No**	Yes
7	2013 – 2014	**Hawkshead (upgraded)**	No	Yes

3.2.3 Quantification of sediment phosphorus flux

Annual net sediment phosphorus flux (kg) was quantified for each year of the study period using a mass balance approach. First, water column phosphorus concentration at the start of each month (TP_{start}, µg l^{-1}) was calculated by averaging all measurements of TP concentration recorded 15 days either side of the first day of the month (usually two measurements, one per fortnight). TP_{start} was then multiplied by lake volume (6.7 x 10^9 l) to obtain an estimate of in-lake phosphorus mass. Changes in lake volume were not taken into account. The difference in starting phosphorus mass between adjacent months was calculated. Changes in phosphorus mass were accounted for by phosphorus input from external sources and the export of phosphorus via the outflow. Unaccounted-for change in phosphorus mass between months was attributed to sediment phosphorus flux:

$$\text{Sediment Phosphorus Flux} = \left(M_{\text{TP\_2}} - M_{\text{TP\_1}}\right) - \left(TP_{\text{in}} - TP_{\text{out}}\right) \qquad (3.7)$$

$M_{\text{TP\_1}}$ is the phosphorus mass (kg) in the lake at the start of the month of interest and $M_{\text{TP\_2}}$ is the mass at the start of the following month. TP_{in} and TP_{out} are the input and outflow loads (kg), respectively. Sediment phosphorus flux was calculated for individual months from January to April and October to December each year. Between May and September Esthwaite Water is typically thermally stratified (George & Hewitt 1999; Maberly *et al.* 2011). As TP concentration is measured in a sample of surface water, it will not be representative of the entire water column when the lake is stratified. To avoid the use of inaccurate phosphorus mass estimates, sediment phosphorus flux was calculated for the whole of the stratified period rather than for individual months, with the May phosphorus mass used as $M_{\text{TP\_1}}$ and October mass used as $M_{\text{TP\_2}}$. Monthly external and outflow loads were combined from May to September each year for use in the calculation of sediment phosphorus flux during stratification.

Sediment phosphorus fluxes for individual months and stratified periods were summed by year to obtain a long-term (1970 to 2014) record of the annual net sediment phosphorus flux. Positive annual net sediment phosphorus flux signifies that the mass of phosphorus released from the sediment to the water column exceeds phosphorus sedimentation. Consequently, negative annual net flux is indicative of a greater mass of phosphorus being incorporated into lake sediment rather than released from sediments over the course of a year. Sediment phosphorus flux was combined with the external phosphorus loads to obtain annual phosphorus budgets for Esthwaite Water.

3.2.4 Water quality response variables and potential driving variables

The responses of two phytoplankton indicators of water quality were assessed in response to variations in indicators of environmental change. The first phytoplankton indicator, chlorophyll *a* concentration, is a widely-used proxy for phytoplankton biomass and has long been used to represent the ecological status of a lake with respect to eutrophication pressures (e.g. European Union 2000; OECD 1982). The second indicator was relative cyanobacterial abundance, reported as the percentage of total phytoplankton biovolume accounted for by cyanobacteria (% cyanobacteria). CEH are in the process of developing a database of phytoplankton cell biovolumes. Using this database (unpublished) and published estimates (Brierley *et al.* 2007; Reynolds 2006), cellular biovolumes were available for 90 of the most frequently counted species in samples from Esthwaite Water. Using these data, phytoplankton biovolumes were calculated for each sampling date throughout the study period from which the percentage of total abundance accounted for by cyanobacteria could be derived. The ratio of chlorophyll *a* concentration to water column TP concentration (Chl:TP) was also included in the statistical analyses as a response variable. The Chl:TP relationship is often used as a measure of productivity per unit TP, as a result of other factors limiting phytoplankton response to phosphorus availability (Chow-Fraser *et al.* 1994; McCauley *et al.* 1989; Spears *et al.* 2013a; Wagner *et al.* 2011). For example, if Chl:TP is relatively low, it is likely that factors other than phosphorus are limiting the accumulation of phytoplankton biomass in a lake. It can therefore be used to predict the likely response of phytoplankton to variations in phosphorus availability.

A range of physico-chemical and biological variables were included in the statistical analyses as potential drivers of water quality in Esthwaite Water. Phosphorus availability, often represented by water column TP concentration, is traditionally considered the key determining factor for phytoplankton biomass (OECD 1982; Schindler 1977; Vollenweider 1976). It has been identified as the main limiting nutrient in previous studies of Esthwaite Water (Maberly *et al.* 2011; Talling & Heaney 1983), hence why it was selected for use in this study to investigate the bottom-up impact of nutrient availability. Water column TP concentration is determined by the balance between external phosphorus load, phosphorus removal via the outflow, and phosphorus flux between the water column and sediment. In this study, the individual effects of external phosphorus load and sediment phosphorus flux on ecological condition were of interest. Therefore, external phosphorus load and water column TP concentration were combined as a single factor to obtain a measure of phosphorus availability that excluded the release

and uptake of phosphorus by lake sediments. This metric, referred to as baseline TP (TP_{base}, µg l^{-1}), was calculated for each month during the study period as follows:

$$TP_{\text{base}} = TP_{\text{start}} + TP_{\text{ret}} \quad (3.8)$$

where, TP_{start} is the water column TP concentration (µg l^{-1}) at the start of the month. Phosphorus retention (TP_{ret}) was calculated by subtracting phosphorus lost via the outflow (TP_{out}, kg) from the external phosphorus load (TP_{in}, kg). The retained phosphorus mass was then converted to volumetric change (µg l^{-1}) for use in the calculation. In combining external load and in-lake TP concentration, co-linearity between two driving variables (external load and water column TP concentration) in the subsequent statistical analyses was avoided. The influence of sediment phosphorus flux on water quality was investigated separately (refer to Section 3.2.7).

The potential top-down impact of grazing pressure on phytoplankton biomass and community composition was investigated by the inclusion of zooplankton filter paper counts (l^{-1}) and trout stocking as driving variables. It is estimated that between 7,500 and 11,000 trout have been released to Esthwaite Water annually since 1982 for game fishing (D. Coleman of Esthwaite Water Trout Fishery, pers. comm., 2015). Unfortunately, a detailed long-term record of trout release and recapture was not available, making it impossible to quantify the stocked trout population in the lake at any one time. As such, trout stocking was treated as a categorical rather than numerical variable (i.e. no stocking 1970 to 1980; stocking 1981 to 2009).

Air temperature (°C) was used to investigate the impact of weather and climate change on water quality. Two other climatic variables were also included; rainfall (mm) as a proxy for hydraulic flushing rate (Jones & Elliott 2007; Reynolds & Lund 1988), and wind speed cubed ($m^3 s^{-3}$), a proxy for wave-mixed depth (Fischer *et al.* 1979). The raw data for each of these variables was averaged by month (January 1970 to December 2014) to remove inherent biases caused by among-season variation in the regularity of sampling or recording.

3.2.5 Trend analysis and identification of turning points

Generalized additive models (GAMs) were used to investigate changes in the long-term trends of water quality response variables and potential driving variables. For each variable, annual means and annual growing season means (May to September, inclusive) were calculated from the monthly mean time series data. GAMs were fitted to these data using the mgcv package (Wood 2017) in R version 3.2.3 for Windows (R Core Team 2016).

GAMs are a non-parametric extension of generalized linear models (GLMs) in which a smoothly varying function is fitted to the data with respect to a predictor covariate (Hastie & Tibshirani 1990). In this case, the predictor covariate was a running year value continuously increasing throughout the monitoring period, fitted with a log-link function and gamma distribution. Compared to GLMs, GAMs are an unrestrictive modelling tool as they allow for a range of trends to be fitted to the time series data, from a straight line, through a range of nonparametric curves of increasing complexity, to an unconstrained piecewise linear fit. The extent of smoothing applied to the fitted curve is a user-defined number of degrees of freedom (d.f.). To best represent overall trends in the data without retaining excessive inter-annual variability, d.f. was set at 0.3 times the number of years in the time series, as recommended by Fewster *et al*. (2000).

From this fitted smoother, the nature of the trend was determined at all points along the time series using the method of Burthe *et al*. (2016). The trend was characterised as being either increasing, decreasing or stationary. To assign any point along the temporal axis to one of these three states, the first derivative of the fitted smoother with respect to time was calculated using finite differences. The standard errors of these derivatives were estimated to provide 95 % pointwise confidence intervals around the gradient and hence assess whether the gradient was significantly different from zero. If the gradient was non-significant, the trend was classified as stationary; otherwise, the trend was classified according to the sign of the gradient. Years in which the trend switched from one state to another were identified as turning-points.

The changes to WwTW and fishery management have been used to define seven management regimes at Esthwaite Water during the study period (Table 3.1). The timings of these regime changes were compared to turning-points identified for the water quality response variables to assess the effectiveness of nutrient management efforts.

3.2.6 Identification of water quality driving variables

The effects of potential driving variables on water quality response variables were analysed using linear mixed-effects models (LMMs). Unlike simple regression analysis, linear mixed-effects modelling allows the nested and hierarchical data structure of many environmental variables to be acknowledged (Zuur *et al.* 2009). This is achieved by the inclusion of both driving variables, known as fixed effects, and random effects. Whereas fixed effects lead to descriptions of mean or "typical" level responses, as in a conventional regression model, the random effects account for nesting in data and the natural

heterogeneity in responses between levels of categorical variables. In this study, global LMMs were constructed for each water quality response variable, including all the potential driving variables as fixed effects. As the time series data were at monthly resolution, sampling month was included as the random effect (slopes and intercepts). This accounted for sub-annual variability in the response of water quality variables to environmental change by determining a different driver-response relationship for each month. These analyses were conducted using the lmer function of the lme4 package for R (Bates *et al.* 2017). The likelihood of obtaining a significant correlation between variables due to their shared seasonality rather than cause-effect relationship was reduced by using the residuals of polynomial regression fits to monthly mean data, thus removing the seasonal component of the time series. LMM assumptions were tested using histograms of residual values, Q-Q plots and plots of residual versus fitted values. When model assumptions were not satisfied, response variable input data were log-transformed to improve the normality and homogeneity of residuals. The statistical significance (i.e. *p* value) of each driving variable included in the LMM was quantified using the summary function.

The dredge function of the MuMIn package (Bartoń 2015) was used to select the combination of driving variables that best explained water quality variability over the monitoring period. Models were compared using Akaike information criterion adjusted for small sample size (AICc). AICc is a measure of the relative quality of statistical models based on the compromise between goodness-of-fit and complexity of parameterization. The model with the lowest AICc was considered to exhibit the best performance of the set tested. Other models with AICc values within four of this "top" model receive similar levels of support from that data and were therefore selected as a confidence set (Burnham & Anderson 2004). The appearance of a driving variable in all confidence set models was interpreted as strong evidence of an effect. In contrast, the appearance of a driving variable in only a portion of models in the confidence set was construed as weaker support for an effect. Importance values, which represents the probability of a variable being included in the best-performing model, were calculated in MuMIn using the relative Akaike weights of models within the confidence set.

As well as conducting the above analysis using monthly data for the whole year, it was also carried-out using just the May to September data. This was done to assess whether the variables determined to be significant drivers of water quality at the annual scale were

also important at the seasonal scale, specifically during the main phytoplankton growing season.

3.2.7 Impact of sediment phosphorus flux on phytoplankton chlorophyll *a* concentration

Predictions of water column chlorophyll *a* concentration were made for a hypothetical version of Esthwaite Water where external phosphorus loading and outflow nutrient export were unchanged, but the annual net sediment phosphorus flux was zero. The prediction was based on the efficiency of phosphorus conversion to phytoplankton biomass (*Chl:TP* ratio) and baseline TP concentration (refer to Equation 3.8). For each year of the study period, annual mean chlorophyll *a* concentration was predicted for the hypothetical lake using the annual mean values of these two variables:

$$\text{Predicted Chlorophyll } a = Chl{:}TP \times TP_{\text{base}} \quad (3.9)$$

The baseline phosphorus metric (TP_{base}) was formulated to represent water column TP concentration if phosphorus flux across the sediment-water interface did not occur. Following this logic, discrepancies between predicted chlorophyll *a* concentrations and the actual annual mean concentrations recorded for Esthwaite Water were attributed to the annual net uptake or release of phosphorus by lake sediments. The difference between real and predicted annual mean chlorophyll *a* concentrations was calculated for each year of the study period (ΔChl, real minus predicted values). The correlation between ΔChl and sediment phosphorus flux was then assessed by Pearson product-moment correlation coefficient. A strong correlation between the two variables was interpreted as sediment phosphorus flux having a significant effect on phytoplankton chlorophyll *a* concentration.

3.3 Results

3.3.1 Phosphorus budget

Average annual contributions of phosphorus sources and sinks (i.e. sedimentation of phosphorus) are illustrated in Figure 3.1. Although there was some change in the apportionment of phosphorus load to Esthwaite Water between Management Regime One (1970 to 1973) and Regime Two (1974 to 1980), the total external phosphorus load did not change considerably (*c.* 1290 kg y^{-1}). With the installation of salmonid culture cages to the lake in 1981, and the consequent introduction of phosphorus from this source, the average annual load increased notably throughout Regime Three (1981 to 1986). The sum of average annual external phosphorus loads was greater for Management Regime Three than for any other management regime. The external phosphorus load decreased steadily from this point to the end of the study period, between Regime Four and Regime Seven.

Upgrades to sewage treatment facilities in 1987 (Regime Three to Regime Four) and 1993 (Regime Four to Regime Five) resulted in large reductions to the phosphorus load from WwTWs, and the removal of the aquaculture cages from the lake in 2009 (Regime Five to Regime Six) resulted in a *c*. 50 % reduction to the total external load. Finally, upgrades to Hawkshead WwTW completed in 2012 (Regime Seven) reduced the sum of annual average external loads to its lowest value since 1970 (562 kg y^{-1}).

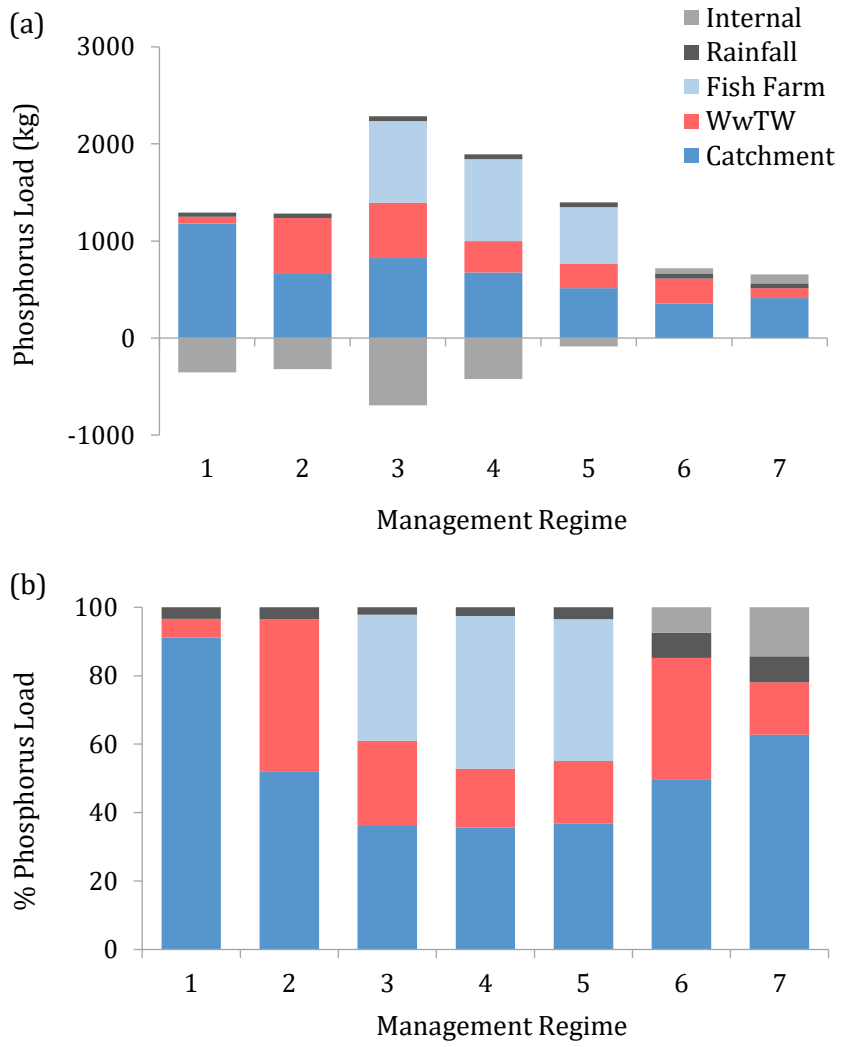

Figure 3.1: Mean annual phosphorus loads to and from Esthwaite Water for each of the seven management regimes between 1970 and 2014 (details in Table 3.1). Values expressed in absolute terms, negative values represent net sediment uptake (a) and as a percentage of phosphorus load (b).

Mass balance derived sediment phosphorus flux was negative for most of the study period, meaning that there was an annual net uptake of phosphorus from the water column by sediments. The period during which the annual mean sediment phosphorus flux was greatest (-694 kg y^{-1}) occurred during Management Regime Three, which coincided with the timing of the highest external phosphorus load. On average, 30 % of the external phosphorus load was retained in the lake sediment during this regime. As external phosphorus loading decreased, so too did the annual uptake of phosphorus by lake sediment. The annual average sediment phosphorus flux was negligible for Management Regime Five (-88 kg y^{-1}). Between 2000 and 2014, the annual release of phosphorus from sediments frequently surpassed the sedimentation of phosphorus, hence introducing an internal load of phosphorus to Esthwaite Water. This was reflected in the positive values of annual mean sediment phosphorus flux calculated for the final two management regimes. By Management Regime Seven (2013 to 2014) internal phosphorus load accounted for 14 % of the total phosphorus load to Esthwaite Water. Most of this load was released during the summer when the external phosphorus load was relatively low and water column chlorophyll *a* concentration was high (Figure 3.2).

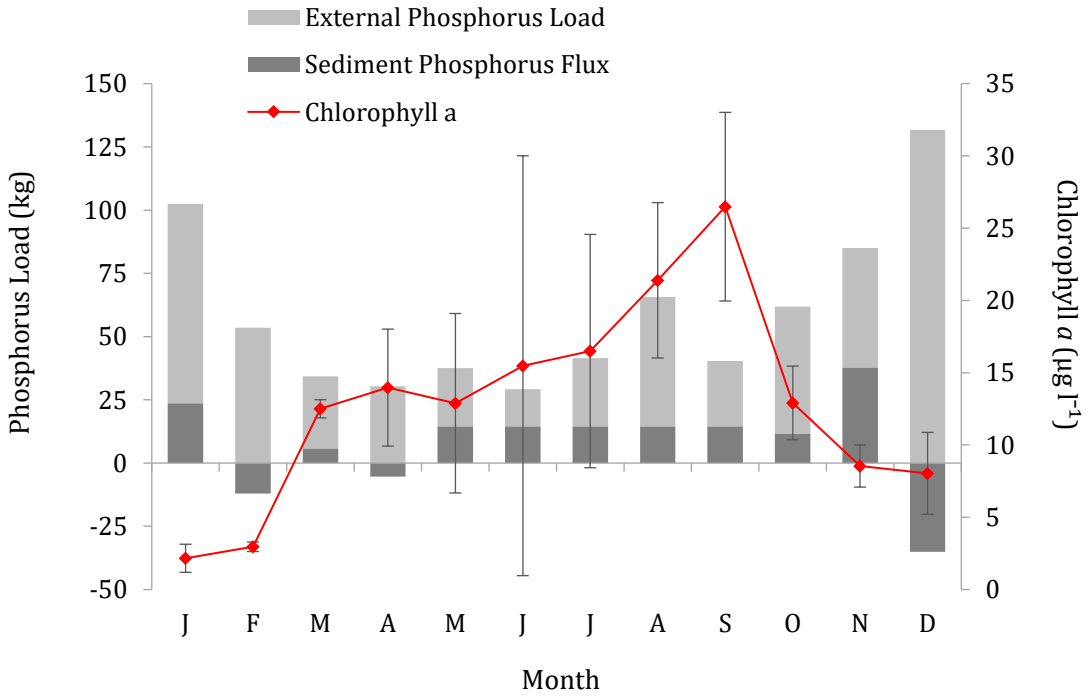

Figure 3.2: Mean monthly external phosphorus load, sediment phosphorus flux and water column chlorophyll *a* concentration of Management Regime Seven (2013 to 2014). The flux calculated for the stratified period is depicted as being evenly distributed between months (May to September). Error bars represent standard deviation of the mean.

3.3.2 Trends and turning points

Of the three water quality response variables tested, the only annual mean time series found to have significant turning points between 1970 and 2014 was the Chl:TP ratio (Figure 3.3). In 1976, the trend switched from stationary to negative. A second turning point was identified in 1978 marking the switch from a decreasing trend back to stationarity. Finally, in 2005 there was a turning point from stationary to positive, with a dramatic increase in the Chl:TP ratio. However, it does not appear that the timing of these turning points coincided with upgrades to local WwTWs or with known changes to aquaculture or fishery operations in the lake. As well as a lack of turning points in the chlorophyll *a* and % cyanobacteria annual mean time series, there were no significant long-term trends determined for either phytoplankton metric. However, the fitted curve for % cyanobacteria did show a slight increasing trend, if not significant, over the study period. When the same analysis was applied to annual growing season means (May to September) none of the response variables were determined to have turning points or significant long-term trends (Figure 3.4).

Significant turning points were detected in the annual mean time series of four out of the five potential water quality driving variables tested (Figure 3.5). Baseline TP concentration increased significantly between 1974 and 1983 then plateaued around 43 $\mu g\ l^{-1}$ until 1997. From 1997 there was a consistent monotonic decline in the annual mean baseline TP concentration. Annual mean rainfall and air temperature both increased significantly throughout the course of the study period. The cube of wind speed was the most undulating of the driving variables with six turning points identified between 1970 and 2014. As with the annual mean time series, the annual growing season mean of baseline TP concentration decreased sharply from the late 1990s until the end of the study period (Figure 3.6). Growing season means of air temperature, rainfall and cube of wind speed did not display any significant trends. The annual growing season mean of zooplankton abundance decreased over the study period, whereas the decline in annual mean was not significant.

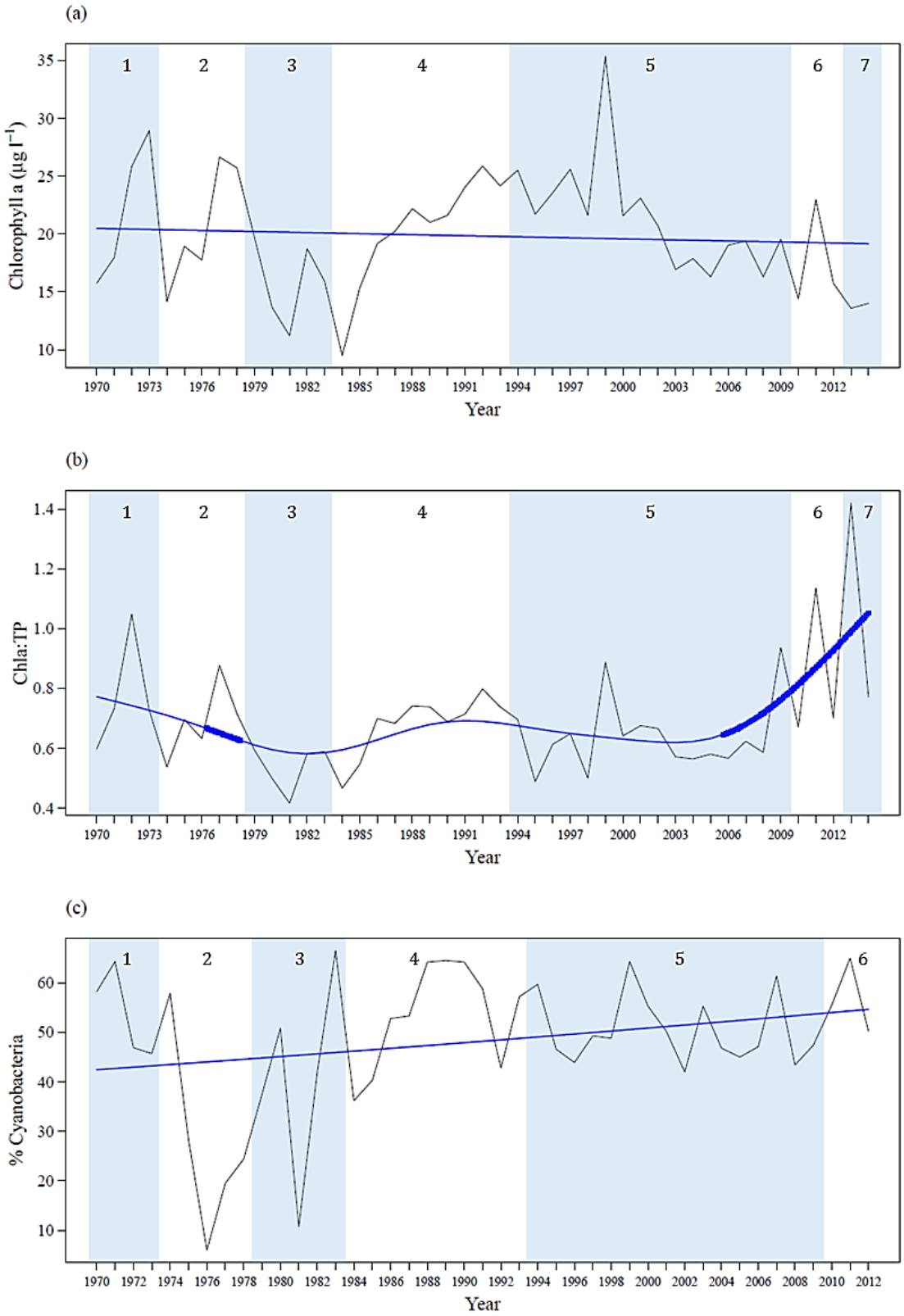

Figure 3.3: Time series plots of annual mean values of water quality indicator variables, 1970 to 2014 (black line). Turning points occur where the fitted curve (blue line) changes thickness. Number labels (1 – 7) correspond to the regimes detailed in Table 3.1. Regime duration and the timing of changes are shown by the shaded bands.

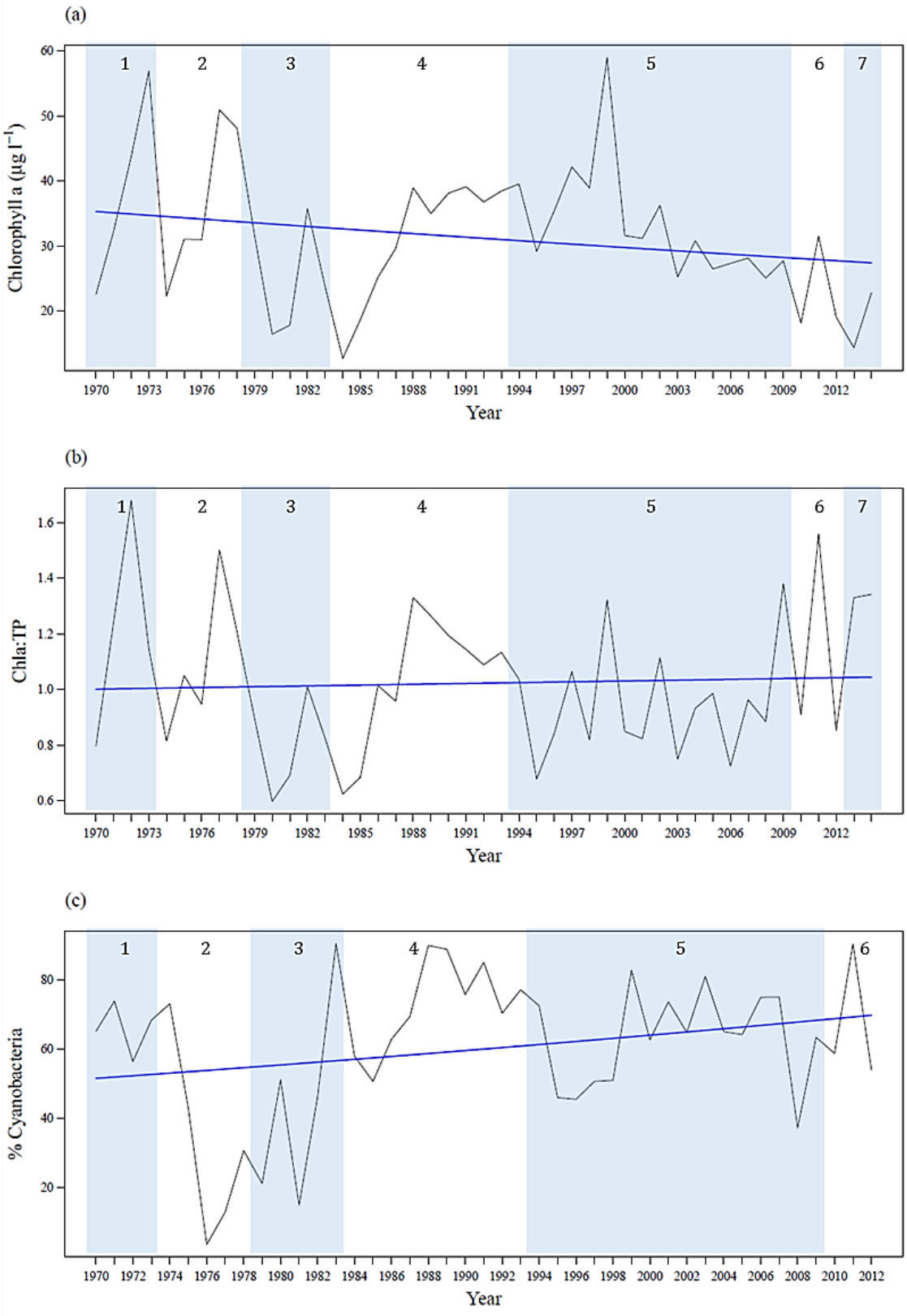

Figure 3.4: Time series plots of annual growing season mean values (May to September, inclusive) of water quality indicator variables, 1970 to 2014 (black line). Turning points occur where the fitted curve (blue line) changes thickness. Number labels (1 – 7) correspond to the regimes detailed in Table 1. Regime duration and the timing of changes are shown by the shaded bands.

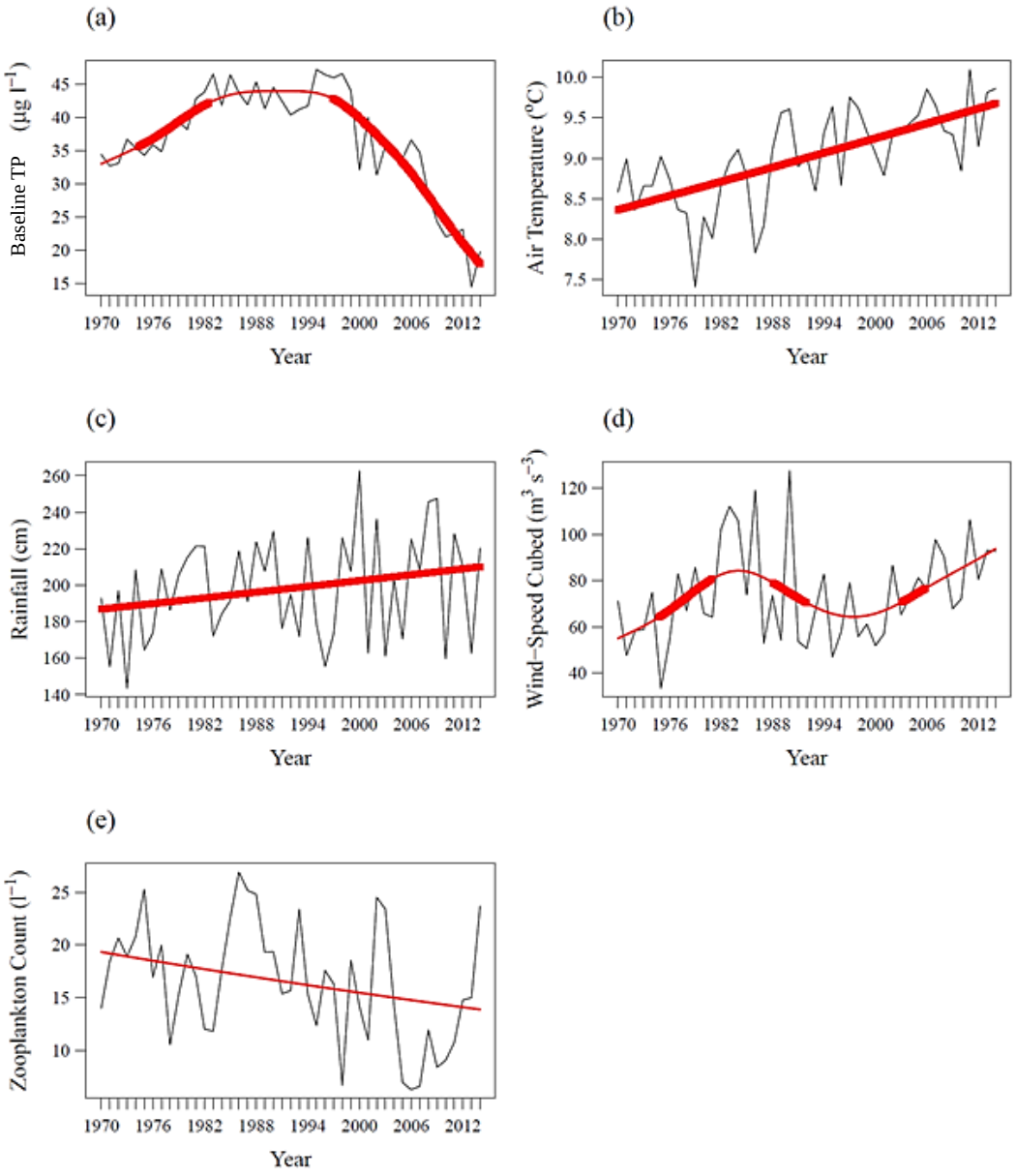

Figure 3.5: Time series plots of annual mean values of potential water quality driving variables, 1970 to 2014 (black line). Turning points occur where the fitted curve (red line) changes thickness.

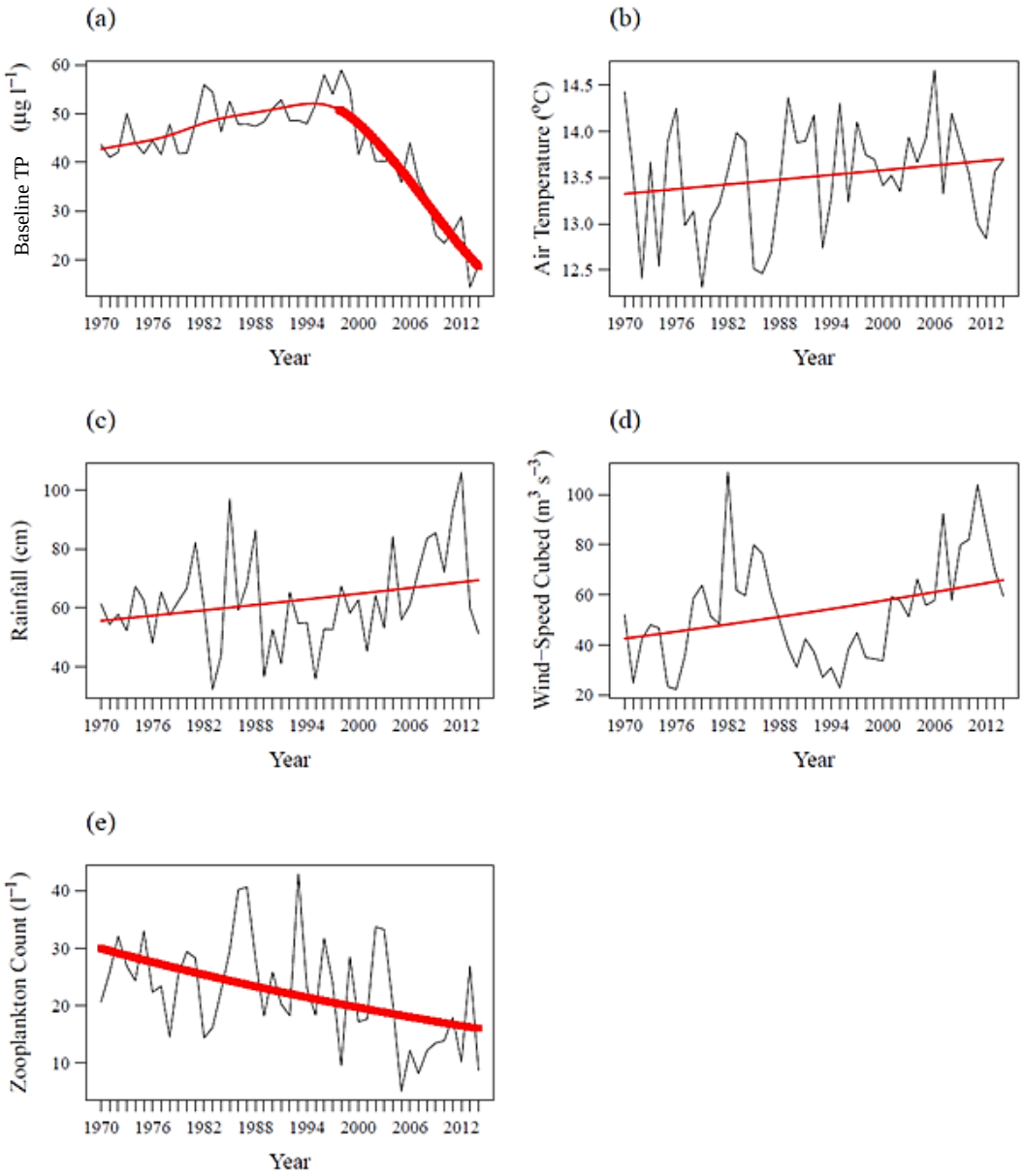

Figure 3.6: Time series plots of annual growing season (May to September, inclusive) mean values of potential water quality driving variables, 1970 to 2014 (black line). Turning points occur where the fitted curve (red line) changes thickness.

3.3.3 Identification of water quality driving variables

Results of the LMMs used to determine drivers of water quality variability at the annual scale are summarised in Table 3.2. Of the six potential driving variables included in the global models, only baseline TP concentration had a significant effect on chlorophyll *a* concentration and Chl:TP ratio. Chlorophyll *a* concentration increased with increasing baseline TP concentration, while the Chl:TP ratio decreased (Figure 3.7a/b). The evidence for any of the other potential water quality driving variables influencing either chlorophyll *a* concentration or Chl:TP ratio is weak. In contrast to chlorophyll *a*, the main drivers of %

cyanobacteria were determined as air temperature and trout stocking (Figure 3.7c/d). Both variables seem to be equally important, appearing in all eight of the confidence set models.

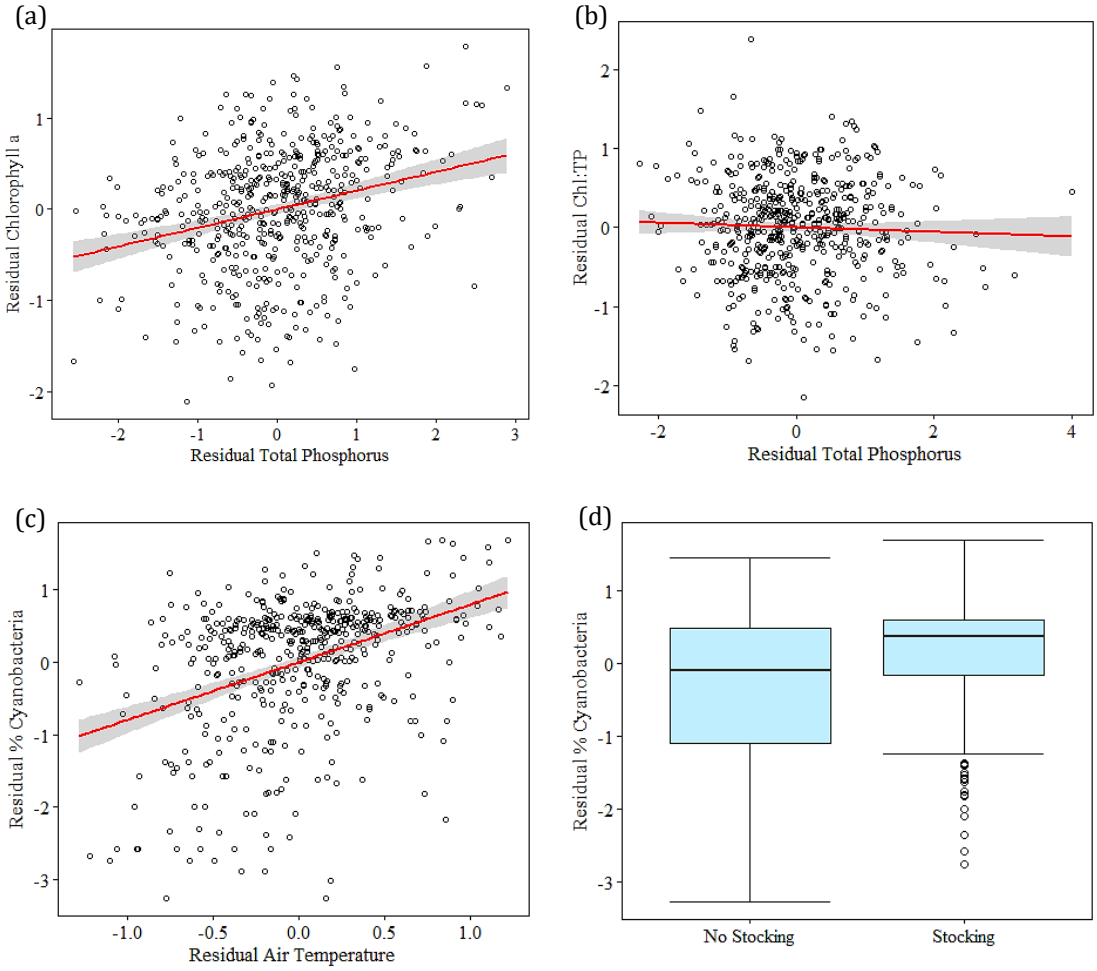

Figure 3.7: Statistically significant relationships between water quality response and driving variables at the annual scale, determined by the top LMMs fitted to residuals of variable monthly mean values. (a – c) 95 % confidence intervals are represented by grey shading. (d) The black line indicates the median, the 25th and 75th percentiles are represented by the box, whiskers show the 10 – 90 % percentiles and outliers are represented as circles.

Table 3.2: Results of the global LMMs used to investigate the effect of potential driving variables on water quality at the annual scale. Statistically significant results are denoted by bold, underscored text ($p < 0.05$). The δAICc ≤ 4 column reports the number of confidence set models in which the driving variable was included. * denotes the inclusion of a variable in the best performing model (lowest AICc). Importance represents the probability of a variable being included in the best-performing model.

Response Variable	Driving Variable	Est.	St. Err.	*t*	*p*	δAICc ≤ 4	Importance
Chlorophyll *a*	Baseline TP	0.13	0.03	4.29	**≤0.01**	17/17 *	1.00
	Air Temperature	0.36	0.10	0.36	0.72	5/17	0.17
	Rainfall	0.01	0.04	0.29	0.78	5/17	0.20
	Cube of Wind Speed	-0.07	0.03	-1.90	0.08	8/17	0.45
	Zooplankton Abundance	-0.04	0.04	-0.82	0.42	4/17	0.17
	Trout Stocking (categorical)	0.22	0.11	1.91	0.08	8/17	0.49
Chl:TP	Baseline TP	-0.10	0.03	-3.53	**≤0.01**	22/22 *	1.00
	Air Temperature	-0.09	0.13	-0.73	0.48	8/22	0.26
	Rainfall	-0.08	0.04	-1.73	0.10	13/22 *	0.62
	Cube of Wind Speed	-0.05	0.04	-1.12	0.29	9/22	0.32
	Zooplankton Abundance	-0.07	0.05	-1.31	0.23	8/22	0.28
	Trout Stocking (categorical)	0.15	0.09	1.63	0.13	11/22	0.45
% Cyanobacteria	Baseline TP	0.08	0.06	1.33	0.20	4/8	0.36
	Air Temperature	0.56	0.14	3.90	**≤0.01**	8/8 *	1.00
	Rainfall	0.03	0.06	0.56	0.58	2/8	0.16
	Cube of Wind Speed	0.02	0.05	0.41	0.69	2/8	0.17
	Zooplankton Abundance	0.07	0.08	0.89	0.40	2/8	0.17
	Trout Stocking (categorical)	0.46	0.10	4.37	**≤0.001**	8/8 *	1.00

Table 3.3: Results of the global LMMs used to investigate the effect of potential driving variables on water quality during the main growing season (May to September, inclusive). Statistically significant results are denoted by bold, underscored text ($p < 0.05$). The δAICc ≤ 4 column reports the number of confidence set models in which the driving variable was included. * denotes the inclusion of a variable in the best performing model (lowest AICc). Importance represents the probability of a variable being included in the best-performing model.

Response Variable	Driving Variable	Est.	St. Err.	*t*	*p*	δAICc ≤ 4	Importance
Chlorophyll *a*	Baseline TP	0.40	0.17	2.28	**0.03**	12/13 *	0.97
	Air Temperature	-2.05	3.10	-0.66	0.51	3/13	0.16
	Rainfall	0.00	0.01	0.19	0.85	2/13	0.12
	Cube of Wind Speed	-0.11	0.09	-1.17	0.25	6/13 *	0.48
	Zooplankton	0.06	0.10	0.59	0.56	4/13	0.20
	Trout Stocking (categorical)	-0.32	3.68	-0.09	0.93	3/13	0.17
Chl:TP	Baseline TP	-0.01	0.00	-2.01	0.05	12/19 *	0.74
	Air Temperature	-0.12	0.08	-1.42	0.16	6/19	0.29
	Rainfall	-0.00	0.00	-0.43	0.67	4/19	0.15
	Cube of Wind Speed	-0.00	0.00	-0.81	0.42	6/19	0.24
	Zooplankton	0.00	0.00	0.54	0.59	5/19	0.19
	Trout Stocking (categorical)	0.03	0.10	0.29	0.77	3/19	0.11
% Cyanobacteria	Baseline TP	-0.00	0.00	-1.73	0.09	6/18	0.32
	Air Temperature	-0.05	0.06	-0.79	0.44	6/18	0.26
	Rainfall	-0.00	0.00	-2.45	**0.02**	12/18	0.67
	Cube of Wind Speed	-0.00	0.00	-0.65	0.52	6/18	0.17
	Zooplankton	-0.00	0.00	-1.44	0.16	8/18	0.43
	Trout Stocking (categorical)	0.24	0.06	3.89	**<0.001**	18/18 *	1.00

The LMMs were repeated using only monthly mean data from the main phytoplankton growing season (May to September). The results of these analyses revealed that the factors controlling water quality varied seasonally (Table 3.3). While baseline TP concentration was a significant driver of chlorophyll *a* concentration during the growing season (Figure 3.8a), it did not have a significant effect on Chl:TP ratio. In fact, none of the six potential driving variables included in the analysis were significant in the Chl:TP global model. Baseline TP concentration was close to having a significant positive effect though and it was the only explanatory variable included in the best performing model. Rainfall, rather than air temperature, was significantly correlated to % cyanobacteria and was included in 12 of the 18 confidence set models. In agreement with the annual data, the evidence for trout stocking as a main driver of variation in % cyanobacteria was strong. Relative cyanobacteria abundance was significantly higher when trout were stocked to the lake than when there was no stocking (Figure 3.8b).

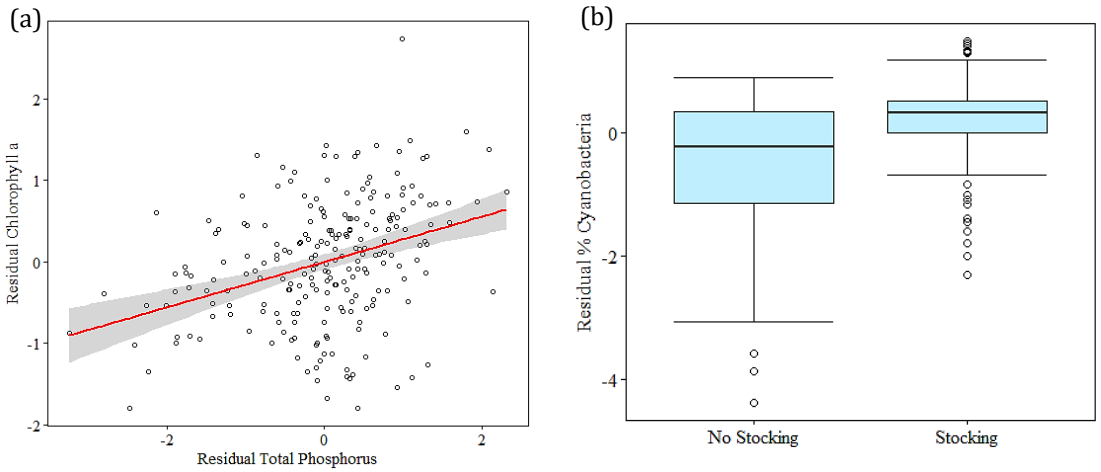

Figure 3.8: Statistically significant relationships between water quality response and driving variables during the growing season (May to September), determined by the top LMMs fitted to residuals of variable monthly mean values. (a – c) 95 % confidence intervals are represented by grey shading. (d) The black line indicates the median, the 25th and 75th percentiles are represented by the box, whiskers show the 10 – 90 % percentiles and outliers are represented as circles.

The difference between real annual mean chlorophyll *a* concentration and predicted chlorophyll *a* concentration decreased throughout the study period (Figure 3.9a). In the mid-1980s the predicted values were up to 18 µg l^{-1} higher than the real measurements of chlorophyll *a*. By 2014 that difference had decreased to 2 µg l^{-1}. ΔChl tended to zero as the external phosphorus load declined. Over the same period the sediment phosphorus flux

transitioned gradually from an annual net uptake of phosphorus by the sediments to a net release. The strong negative correlation between sediment phosphorus flux and ΔChl (r = 0.85, p < 0.001) is indicative of sediment phosphorus flux having an effect on phytoplankton growth (Figure 3.9b).

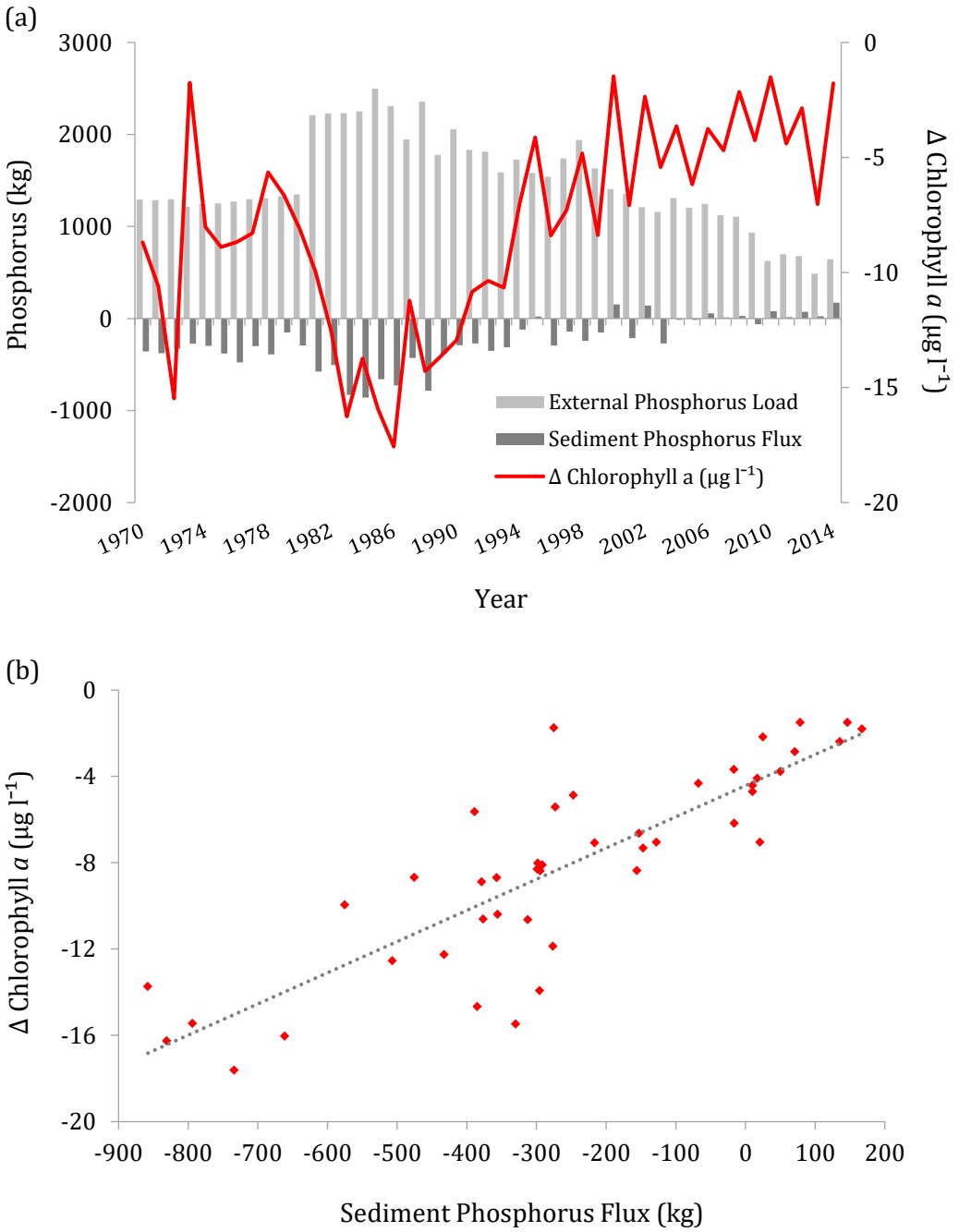

Figure 3.9: (a) Time series plot showing the annual external phosphorus load to Esthwaite Water, annual net sediment phosphorus flux and ΔChl (annual mean of measured chlorophyll *a* minus the annual mean of modelled chlorophyll *a*). (b) The correlative relationship between ΔChl and sediment phosphorus flux.

3.4 Discussion

3.4.1 Phosphorus budget

Anthropogenic activities in Esthwaite Water and its catchment were the cause of considerable variation in the annual lake phosphorus budget between 1970 and 2014. Initially, diffuse phosphorus input from the catchment was the main contributor to the overall phosphorus load. Since then, increasing lake water phosphorus concentrations and water quality deterioration have generally been attributed to two local developments; the establishment of Hawkshead WwTW in 1973 and the installation of aquaculture cages to the lake in 1981 (Hall *et al.* 1993; Maberly *et al.* 2011; Talling & Heaney 1983). However, while Hawkshead WwTW was certainly the source of a sizeable phosphorus load to Esthwaite Water, it seems likely that its net effect in terms of changing the total external phosphorus load was relatively small at first. This is because it replaced numerous septic tanks in the Hawkshead area that had previously overflowed into Black Beck, augmenting diffuse phosphorus inputs from the catchment area (May *et al.* 1997). Unfortunately, there was an absence of Black Beck phosphorus data around the time Hawkshead WwTW was installed with which this theory could have been tested. Even so, it was accounted for by subtracting the estimated phosphorus load for the new WwTW from an earlier estimate of catchment area phosphorus load. Therefore, in the record of external phosphorus load presented here the WwTW load did not immediately increase the total external load to the lake. The installation of salmonid aquaculture cages to Esthwaite Water undoubtedly had a significant and instantaneous impact on the lake phosphorus budget. The flux of uneaten fish food, excrement and sloughed scales from the cages to the surrounding water column effectively doubled the total external phosphorus load. The external phosphorus load peaked in the few years following the start of aquaculture.

One of the advantages of establishing Hawkshead WwTW was that it consolidated a diffuse source of phosphorus to a single point source, which could be relatively easily controlled. From the late 1980s sewage treatment procedures and management at Hawkshead WwTW and Near Sawrey WwTW underwent several changes in an attempt to reduce the external phosphorus load to Esthwaite Water and improve lake water quality (Hall *et al.* 1993). In compiling estimates of WwTW phosphorus load from various short-term studies conducted since then it is apparent that these upgrades were successful (Agar *et al.* 1988; Hall *et al.* 1993). However, given that the WwTWs were relatively minor contributors of phosphorus to the total external load, the decrease in phosphorus load from this source only had a moderate impact on the overall phosphorus budget (Figure 3.1). The external load remained high until the aquaculture cages were

removed in 2009 and there was a complete cessation of phosphorus input to Esthwaite Water from this source. With the final upgrade to Hawkshead WwTW completed in 2012, the total external phosphorus load reverted to being dominated by diffuse runoff. Throughout the study period the catchment phosphorus load decreased. This was likely due to the introduction of the Defra-funded Catchment Sensitive Farming Delivery Initiative, which encourages environmental stewardship through the provision of grants to fund environmentally sensitive farming practices in the region (Hodge & Reader 2010; Natural England 2016). If further reductions to the external load are to be achieved, it will depend on the continued application and improvement of measures to reduce agricultural diffuse pollution.

Results of the mass balance model suggest that as the external phosphorus load decreased, there was a gradual shift from annual net uptake of phosphorus by lake sediments to net release. This long-term pattern from 1970 to 2014 in sediment phosphorus flux is consistent with observations from many other shallow lakes where external phosphorus loading has been reduced (Jeppesen *et al.* 2005; Rossi & Premazzi 1991; Sas 1989; Søndergaard *et al.* 1999; Spears *et al.* 2012). The switch from net downward flux of sediment phosphorus to a net upward flux occurs because phosphorus sedimentation no longer outweighs the gross release of phosphorus from the sediment by a variety of biological and physico-chemical mechanisms, (Søndergaard *et al.* 2001). The importance of various sediment phosphorus mobilisation mechanisms in Esthwaite Water are investigated in Chapter Four. Despite this recently emerged internal load, the overall phosphorus load to Esthwaite Water is greatly reduced and lower than it has been at any time since 1970, the start of this study period.

Annual lake phosphorus budgets are extremely difficult to describe with accuracy and precision (Dillon & Evans 1993; Hall *et al.* 1993). Several potential sources of inaccuracy were identified in the calculation of historical phosphorus budgets for Esthwaite Water. For example, most external sources of phosphorus to Esthwaite Water were not subjected to continuous monitoring. Annual load estimates were available from previous investigations for discrete points throughout the study period. Gaps in the data set were mostly interpolated between these pre-existing load estimates. While general trends may be reasonably well represented by this approach to obtaining long-term records of phosphorus load, it will not reflect inter-annual load variability. It is also possible that significant sources of phosphorus were not identified and therefore not accounted for in the phosphorus budget. For example, phosphorus in excrement from the overwintering

bird population and diffuse phosphorus run-off directly to the lake from surrounding fields. Even the catchment phosphorus load derived from regular measurements of Black Beck TP concentration may be inaccurate. It is well established that upwards of 80 % of annual phosphorus load to a waterbody is transported by just a few high-flow events (Jordan *et al.* 2012; Sharpley *et al.* 2008). Unless routine sampling of Black Beck coincided with such events, the catchment area phosphorus load is likely to be an underestimate (Defew *et al.* 2013). Any inaccuracies in the external phosphorus load would have transmitted to the sediment phosphorus flux calculated by mass balance modelling.

3.4.2 Effect of phosphorus availability on water quality

Baseline TP concentration was identified as the single most important driver of phytoplankton biomass variation in Esthwaite Water, both during the main growing season and annually. As baseline TP was formulated to represent variability in water column TP concentration due to external phosphorus loading, it could be expected that large, abrupt changes in the phosphorus loads from major external sources would be reflected in the long-term chlorophyll *a* record. However, the decrease in chlorophyll *a* concentration was not significant over the course of the 44-year study period and trend analysis did not determine a single turning point that coincided with notable management changes at either the WwTWs or the fish farm. It seems that despite the significant reductions in external phosphorus load, this has not been sufficient to limit phytoplankton growth.

The emergence of the internal phosphorus load appears to be partly responsible for the continuation of poor water quality. Support for this theory comes from phytoplankton modelling. For much of the study period the chlorophyll *a* concentration modelled from baseline TP concentration was considerably higher than the actual annual mean chlorophyll *a* concentration. This difference decreased as the sediment phosphorus flux transferred from a net uptake to a net release, which suggests that early in the study period much of the external phosphorus load to Esthwaite Water was not converted to phytoplankton biomass. Biological phosphorus uptake must have been limited by a factor not accounted for in this study, for example the bioavailability of phosphorus forms, nitrogen availability or light attenuation. The phosphorus load not incorporated into phytoplankton biomass or flushed from the lake, presumably accumulated in the sediment via physico-chemical pathways, e.g. adsorption to iron oxy-hydroxides. Despite reducing external load, phosphorus levels remained excessive in the lake, high phytoplankton biomass was maintained but progressively less phosphorus was accumulated in the

sediment. By the end of the study period the difference between modelled and real chlorophyll *a* concentration was almost zero, indicating that phytoplankton biomass was being maintained at the same high levels by utilising almost all of the phosphorus load.

Since sediment phosphorus flux switched to a net release, much of the phosphorus load was accounted for by internally sourced phosphorus (*c*. 14 %). It is likely that the phosphorus load during the final management regime was more bioavailable than it had been previously. Whereas a significant portion of externally sourced phosphorus is likely to be bound to mineral particles and not bioavailable, phosphorus released from lake sediments is largely in the bioavailable form of soluble reactive phosphorus (Reynolds & Davies 2001). This may also help explain the increase in the Chl:TP ratio, which reflects the greater bioavailability of the total phosphorus load rather than an improvement in growth conditions due to other factors, for example reduced grazing pressure by zooplankton (Chow-Fraser *et al.* 1994). The increased ratio also suggests that phosphorus is becoming more strongly limiting at an annual scale. The same trend in Chl:TP ratio was not detected for the main phytoplankton growing season. Overall, TP concentration was unchanged at this time of year as the internal phosphorus load was sufficient to counteract the reduction in external phosphorus load, which was typically relatively low during the main phytoplankton growing season anyway. Nevertheless, Chl:TP ratio is expected to be linear below water column TP concentrations of *c*. 100 $\mu g\ l^{-1}$ (Janus & Vollenweider 1981), as is the case in Esthwaite Water throughout the whole of the year. Therefore, assuming no other factors are limiting phytoplankton biomass, further reductions in phosphorus load should begin to improve the water quality of Esthwaite Water (Spears *et al.* 2013a).

Upward sediment phosphorus flux has been shown to decrease gradually in other lakes following the reduction of external phosphorus load (Jeppesen *et al.* 2005; Søndergaard *et al.* 2002; Spears *et al.* 2012). However, predicting the duration of this re-equilibration period is challenging as it is influenced by multiple factors, for example loading history, lake flushing rate and chemical characteristics of the sediment (Søndergaard *et al.* 2001). Observations at other sites suggest a re-equilibration period of at least 10 years following external phosphorus load reduction (Marsden 1989; Søndergaard *et al.* 2001; Spears *et al.* 2012). A decrease in the internal phosphorus load to Esthwaite Water over the coming decade might yet produce water quality improvements in line with the EU WFD targets and within the expected timeline. However, as previously mentioned, internal load only accounted for 14 % of the annual total phosphorus load on average during the final management period (2013 to 2014), albeit with a high bioavailability. Water column

phosphorus concentration was still largely dependent on the external load and future improvements in water quality might only be achieved if it is reduced further.

3.4.3 Effect of trout stocking on water quality

It was difficult to make an *a priori* prediction of water quality response to trout stocking in Esthwaite Water as trout are omnivorous. Feeding at multiple trophic levels has the potential to weaken the cascading trophic interactions that would be expected if the introduced fish were either piscivorous or planktivorous (Carpenter *et al.* 1985; Pace *et al.* 1999; Threlkeld 1988). Results of the LMMs revealed that % cyanobacteria was significantly higher when trout were stocked to the lake than when there was no stocking. The interactions or processes that resulted in the % cyanobacteria increase are not clear. The relationship between zooplankton abundance and % cyanobacteria was not determined to be significant, which suggests that the increase was not a consequence of diminished grazing pressure on cyanobacteria due to increased trout predation of zooplankton. It is possible that alterations to a different trophic interaction caused % cyanobacteria increase. For example, Nyström *et al*. (2001) demonstrated a trout-induced trophic cascade in ponds, where rainbow trout reduced the biomass and activity of grazing snails, resulting in higher phytoplankton biomass.

Alternatively, the increased dominance of cyanobacteria may have been a response to changes in bottom-up processes instigated by trout stocking, rather than a response to alteration of top-down control by grazing. It has been proposed that stocked trout fundamentally alter nutrient cycles and stimulate phytoplankton growth by accessing benthic phosphorus sources not usually available to pelagic communities in lakes that were previously fishless (Leavitt *et al.* 1994; Schindler *et al.* 2001). Although Esthwaite Water has a natural fish population, which includes native brown trout, the introduction of 11,000 trout annually is likely to have a significant impact on phosphorus cycling. Excretion of benthic derived phosphorus by fish to a nutrient-depleted epilimnion during periods of stratification would be especially advantageous to nitrogen-fixing cyanobacteria. Further investigation is needed to determine if enhancement of this biotic feedback mechanism caused the % cyanobacteria increase following the start of trout stocking. Confirmation of this scenario would pose a difficult challenge for lake managers charged with balancing the demand for a recreational fishery with the need to improve water quality.

3.4.4 Effect of climatic variables on water quality

While variability in total phytoplankton biomass does not appear to have been driven directly by climatic variables, the proportion of phytoplankton biovolume accounted for by cyanobacteria does. At the annual scale, there was a significant positive relationship between air temperature and % cyanobacteria. However, despite a significant long-term increase in air temperature, there was not a concurrent increase in annual mean % cyanobacteria. Shorter-term inter-annual fluctuations are likely to be responsible for the positive relationship. Long-term studies at several other lakes have revealed an increase in cyanobacterial dominance associated with warming, for example, Lake Müggelsee, Germany (Wagner & Adrian 2011); the Heiligensee, Germany (Adrian *et al.* 1995); Lake Zurich, Switzerland (Posch *et al.* 2012); and Lakes Vänern, Vättern and Mälaren, Sweden (Weyhenmeyer 2001). Although these long-term studies are relatively few, the evidence for a positive effect of temperature on cyanobacteria is strengthened by the findings of laboratory experiments (De Senerpont Domis *et al.* 2007), phytoplankton community modelling experiments (Elliott 2012), studies of the impacts of heat waves (Anneville *et al.* 2015; Jöhnk *et al.* 2008) and comparative studies of lakes across a range of climates (Jeppesen *et al.* 2009; Kosten *et al.* 2012).

These increases in cyanobacteria biomass have been attributed to cyanobacteria being more tolerant of high temperatures than other phytoplankton groups (Butterwick *et al.* 2004). Additionally, increased strength and duration of stratification and the consequent reduction in vertical mixing, which enhances the scope for buoyancy controlled vertical positioning of cyanobacteria, may be an important factor (Paerl *et al.* 2011; Wagner & Adrian 2009). Under stable water column conditions, positively buoyant cyanobacteria will be able to regulate their position in the water column and remain in the euphotic zone, while non-motile, negatively buoyant algae that can only remain suspended in the water column under mixing conditions, such as diatoms, will tend to sink (Reynolds 1984a). Nitrogen-fixing cyanobacteria may also have an advantage over an extended growing season as species unable to fix atmospheric nitrogen may decline in surface waters if dissolved inorganic nitrogen becomes depleted. The reason why cyanobacteria have not become more dominant in Esthwaite Water despite the apparent improvement in growing conditions will require further study, but it might relate to phosphorus availability or the already large proportional abundance of this group of phytoplankton during the summer growing season.

Although increasing air temperature does not appear to have boosted the proportional abundance of cyanobacteria, the increasingly favourable growing conditions due to warming may be responsible for their continued dominance, and indirectly for the maintenance of high overall phytoplankton biomass. Greater improvements in water quality may already have been observed at Esthwaite Water had climate warming not occurred simultaneously with phosphorus load reductions. The threat posed to ecosystem and human health by high cyanobacteria abundance in lakes means that it is imperative that the effects of climate change are mitigated (Codd *et al.* 2005).

It is thought that there is a synergistic effect between nutrient availability and climate change, meaning that the effects of warming on phytoplankton communities are enhanced in lakes of a higher trophic state (Kosten *et al.* 2012; Paerl *et al.* 2011; Paerl & Huisman 2008). It has been suggested that control of nutrient availability to reduce the effects of eutrophication will also diminish the impacts of climate change (Moss *et al.* 2011). There was only moderate support for phosphorus (baseline TP concentration) as a driver of annual mean % cyanobacteria in Esthwaite Water. Nevertheless, Esthwaite Water appears to be heading towards a phase of stronger phosphorus limitation and further phosphorus load reductions are expected to reduce overall phytoplankton biomass, regardless of community composition. However, it is unclear from these data the magnitude of reductions required to reduce cyanobacterial dominance and improve water quality in the face of ongoing climate change. This issue is investigated further in Chapter Five.

Rainfall had a negative effect on % cyanobacteria during the growing season. Rainfall was used as a proxy for flushing rate. The negative relationship between the two variables is in keeping with the perceived view that cyanobacteria are not suited to rapidly flushed lakes as cells can be removed from the system more quickly than the slow growing cyanobacteria can replace them (Reynolds 2006; Vollenweider & Kerekes 1980). These findings also validate modelled predictions of cyanobacteria response to flushing rate variations in Esthwaite Water (Elliott 2010). Manipulation of the watershed hydrological system to increase flow through lakes could be used to inhibit the formation of cyanobacterial blooms. However, this approach to control the effects of eutrophication and/or climate warming is logistically difficult, potentially costly and is dependent on low nutrient content water for flushing, so as not to worsen enrichment in eutrophic lakes (Paerl 2014; Qin *et al.* 2010).

3.4.5 Conclusion

Monitoring data collected from Esthwaite Water over a period of four decades, which encompassed significant environmental change both at a local and global scale, were analysed to determine potential drivers of water quality variability. Although phosphorus availability was identified as the most important driver of phytoplankton biomass, significant changes in biomass did not coincide with abrupt alterations to the external phosphorus load resulting from anthropogenic activity in the lake and catchment area. Furthermore, despite substantial reductions to the external phosphorus load in recent years a significant improvement in water quality was not observed. As hypothesised, it appears that the emergence of phosphorus release from lake sediments and the effects of climate change on phytoplankton community structure buffered against the desired impact of reduced external nutrient loads. Further reduction of the external phosphorus load, specifically diffuse phosphorus emissions from the catchment, or the implementation of measures to inhibit sediment phosphorus release may be sufficient to counter the effects of high historical nutrient loading and ongoing climate change on water quality. The implications of these analyses for the future management of Esthwaite Water and other similarly impacted lakes will be discussed in greater detail in the General Discussion (Chapter Six).

Chapter Four

Impact of salmonid cage aquaculture on phosphorus content and fractionation in lake sediments: A case study in Esthwaite Water, UK

In the previous chapter internal phosphorus loading was found to contribute towards the maintenance of high phytoplankton biomass in Esthwaite Water following reductions to the external phosphorus load. This chapter investigates contemporary sediment phosphorus distribution, inventory, mobility and release mechanisms in relation to the site of historical fish farm operations.

4.1 Introduction

Aquaculture provides an affordable and much-needed source of high-quality animal protein for human consumption (AFSPAN 2015; Tidwell & Allan 2001). Global aquacultural finfish production has increased rapidly, from 1.5 million tonnes in 1970 to 73.8 million tonnes in 2014 (FAO 2016). In line with the global trend, the UK freshwater and marine aquacultural industries have also expanded over the same period to keep-up with increasing demand (Seafish 2016). Species cultured in the UK largely comprise salmonids including rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*) and Atlantic salmon (*Salmo salar*). Two main types of production system are used for salmonids; floating cage systems in natural freshwater or coastal marine areas, and inland artificial ponds (CEFAS 2012; Marine Scotland 2015). A recent evaluation of the UK's freshwater fisheries estimated that they support about £1 billion of our household income, the equivalent of 37,000 jobs (Defra 2015). Although the industry is undoubtedly of great socio-economic importance, the negative environmental impacts posed by aquaculture practices are numerous. They include the introduction of non-native species (Fleming *et al.* 2000; Hutchings 1991; McGinnity *et al.* 1997), nutrient enrichment of the host waterbody (Bureau & Hua 2010; Kelly 1993; Yan 2005), transmission of parasites and pathogens to wild fish populations (Austin & Austin 2007; Krkošek *et al.* 2007), and dispersion of antibiotics (Cabello 2006; Sapkota *et al.* 2008). In addition to ecosystem degradation, the introduction of such stressors could compromise the sustainability of the aquacultural industry itself, which relies on the supply of high-quality water (Phillips *et al.* 1985).

Rearing fish in an intensive manner involves the transformation of dietary inputs into fish biomass. This process generates wastes, which are, in many cases, difficult to contain and recover. Of major concern in freshwater ecosystems is the release of phosphorus containing compounds from fish culture cages in forms that may stimulate primary productivity (phytoplankton growth) and eutrophication processes in the receiving waterbody (Reynolds 1984b; Schindler 1977). It has been estimated that as little as 17 % of phosphorus introduced to fish culture cages in pelletised feed is retained in the fish biomass and subsequently removed with the harvest (Holby & Hall 1991). The remainder is lost to the water column via excreta, sloughed scales and mucus, uneaten pelleted feed and fish mortalities. Depending on its magnitude, the introduced phosphorus load may trigger a series of undesirable symptomatic changes in the host waterbody, which can ultimately cause an increase in phytoplankton biomass leading to the loss of macrophytes, especially in shallow lakes (Scheffer *et al.* 1993, 2001).

Significant efforts of stakeholders in the aquaculture industry have resulted in a reduction of waste outputs from fish culture cages, particularly in salmonid production operations (Bureau & Hua 2010). Enhanced feed digestibility and nutrient utilisation efficiency have been achieved through the selection of better ingredients, improved feed formulation and the use of various feed additives, such as enzymes (NRC 2011). Recent technological advances also allow for the swifter removal of fish mortalities from the cages. As a result of these efforts, nutrient release from finfish aquaculture, in both marine and fresh waters, has risen less rapidly since the 1970s than fish production (Bouwman *et al.* 2013). However, regardless of these improvements, the eutrophication of aquaculture may only be reversed or prevented in some host waterbodies by the complete removal of culture cages from the system; especially if legislative targets of water and ecological quality, for example, those set by the EU Water Framework Directive (WFD), are to be achieved.

A substantial quantity of the phosphorus released from cages will accumulate in lake sediments, either directly by sedimenting particulate waste or indirectly via assimilation into the local food web and the subsequent sedimentation of organic matter (Beveridge 1987; Gondwe *et al.* 2012; Hall 1991; Sarà *et al.* 2004). Numerous studies in the marine environment and a few in freshwater systems have reported elevated phosphorus concentrations in the surface sediment below culture cages (Cornel & Whoriskey 1993; Holby & Hall 1991; Karakassis *et al.* 1999; Kelly 1993; Wu *et al.* 1994). The accumulation of phosphorus in sediments, whether it be from fish farming or some other external source (e.g. treated sewage effluent, agriculture), can have long-term consequences for the management of eutrophic lakes. It is generally accepted that the release of phosphorus from enriched sediments to the water column (referred to as internal loading) is responsible for the delay in expected water quality improvements observed in many lakes following the reduction of external nutrient loads (Phillips *et al.* 1994; Sas 1989; Søndergaard *et al.* 2007; Spears *et al.* 2012). Crucially, this mechanism can prevent water quality and ecological targets being met within the timeframes set out by environmental legislation (Lang *et al.* 2016; Spears *et al.* 2007a). However, the maintenance of internal loading is ultimately dependent upon the external phosphorus supply and will, therefore, reduce over time if the external load remains low (Lijklema 1985; Marsden 1989).

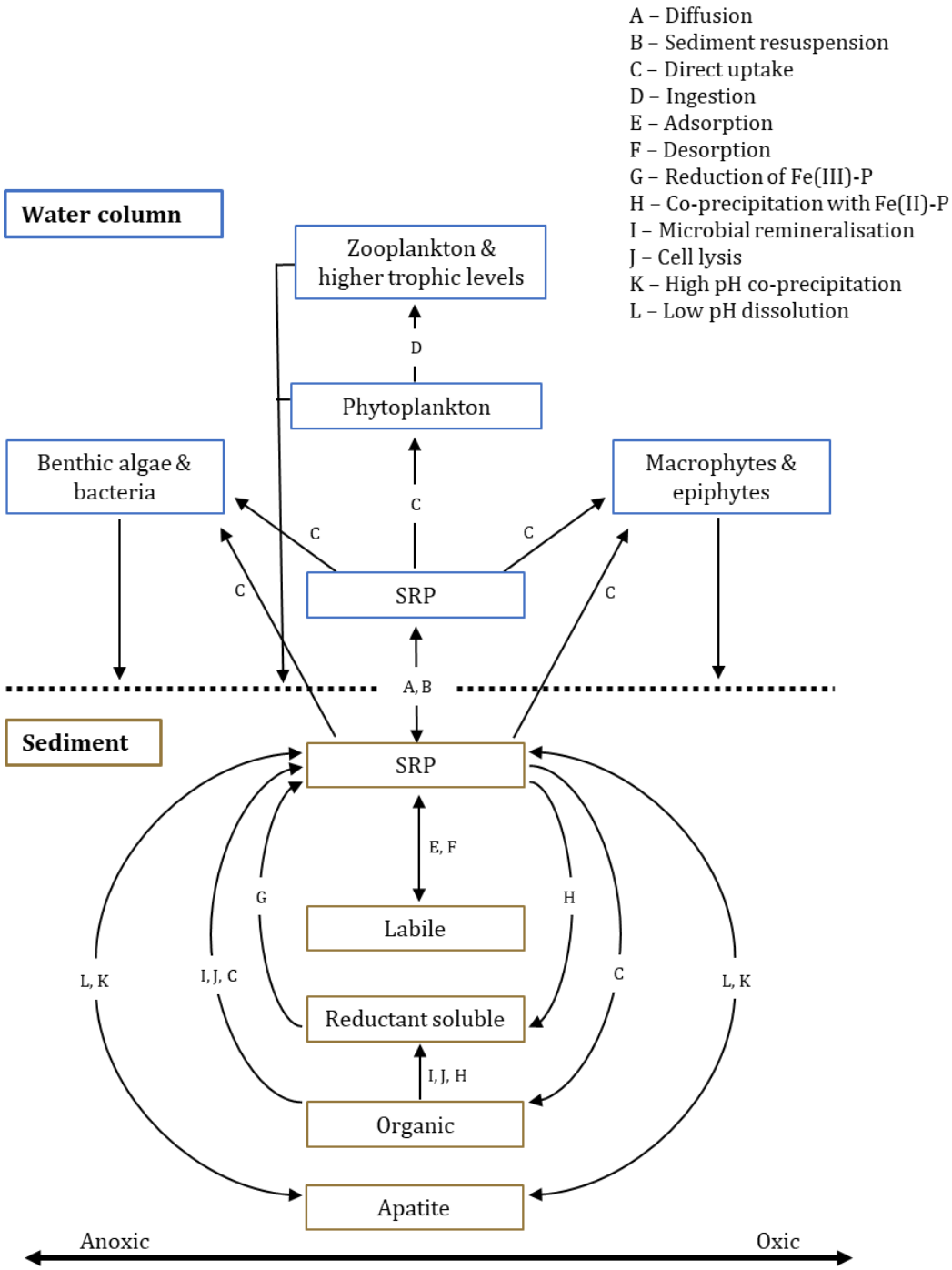

Figure 4.1: Pathways of phosphorus transfer across the sediment-water interface under oxic and anoxic conditions at the sediment surface. Adapted from Spears *et al.* (2007a).

The total pool of phosphorus in the sediment can be partitioned between several operationally defined fractions: labile phosphorus (e.g. dissolved in pore-water or loosely adsorbed to sediment); reductant-soluble phosphorus (e.g. bound to Fe-oxyhydroxides and Mn-compounds); organic phosphorus (e.g. contained within detritus); metal-oxide adsorbed phosphorus; apatite-bound phosphorus; and refractory phosphorus

(Pettersson *et al.* 1988; Psenner *et al.* 1984). As illustrated in Figure 4.1, the mobility of phosphorus across the sediment-water interface and between fractions is determined by an interplay of physico-chemical variables. These include temperature, pH, redox condition, concentration gradients, wind-induced sediment resuspension and biological factors, for example, microbial mineralisation, benthic macro-algae and macrophyte cover and growth, and bioturbation (Boström *et al.* 1982; Jensen & Andersen 1992; Mortimer 1941, 1942; Spears *et al.* 2006; Welch & Cooke 1995). Recent studies indicate that cage aquaculture not only increases the quantity of phosphorus in below-cage sediments but also influences its distribution between these different fractions (Jia *et al.* 2015; Matijević *et al.* 2008, 2012).

Data from Esthwaite Water, a shallow lake in which salmonid aquaculture cages were formerly installed, were used to address the following study objectives:

(i) Test the hypothesis that the installation of aquaculture cages in a lake increases sediment phosphorus concentration and alters sediment phosphorus fractionation relative to an unimpacted control site;

(ii) Determine if a legacy of high sediment phosphorus concentration persists following the removal of fish culture cages;

(iii) Determine the potential mechanisms that control sediment phosphorus release at a former fish cage site; and

(iv) Assess the implications that sediment phosphorus enrichment from aquacultural waste could have on lake remediation and recovery from eutrophication.

4.2 Methods

4.2.1 Study site

The study was conducted at Esthwaite Water, a shallow, naturally eutrophic lake in the English Lake District (Cumbria, UK). Throughout the last century, Esthwaite Water has been subject to high external nutrient loading from a variety of anthropogenic sources, including from rainbow trout culture cages installed in the central impact basin of the lake from 1981. The fish farm produced approximately 100 tonnes of rainbow trout per annum. Hall *et al.* (1993) estimated the total phosphorus (TP) and soluble reactive phosphorus (SRP) loads from the cages to the water column at 812 kg y^{-1} and 284 kg y^{-1}, respectively. The introduction of phosphorus from aquaculture contributed to the maintenance of high algal biomass in Esthwaite Water despite reductions made from other sources during its operation. In recent years, significant efforts have been made to reduce the phosphorus concentration and phytoplankton biomass of the lake in order to

achieve EU WFD Good Ecological Status. Of most relevance to this study, the fish cages were removed in 2009 resulting in the complete cessation of the input phosphorus flux from this source. Additionally, a nearby waste water treatment facility that discharges into the main inflow, underwent extensive upgrades between 2011 and 2012 to reduce the phosphorus load reaching the lake via treated sewage effluent. Refer to Chapter Three for further details of the impact of fish farming and waste water treatment operations on lake water quality.

4.2.2 Historical sediment phosphorus data

Between March 1986 and June 2001, surface sediments from 12 locations across the lake were sampled regularly and the concentration of alkali-extractable phosphorus (AEP) determined (Hall *et al.* 2001). Sampling locations are shown in Figure 4.2. AEP is also known as mobile phosphorus and comprises labile phosphorus, reductant-soluble phosphorus and organic phosphorus. The sum of these fractions is considered to represent the release-sensitive phosphorus pool (Boström *et al.* 1982; Søndergaard *et al.* 2003). These data allow the current sediment phosphorus concentrations to be compared with the sediment phosphorus concentrations when the fish farm was in operation.

4.2.3 Field sampling

Fieldwork was conducted monthly throughout 2014. Nine sampling sites were selected in each basin, the northern control basin and the central impact basin, to span a wide range of water depths and provide good spatial coverage (Figure 4.2). It was important to select control sampling sites that were sufficiently isolated from the influence of the fish farm, but also close enough to the impact sites to be subject to the same range of phenomena (e.g. weather, climate change, external nutrient loading) that drive long-term and seasonal changes in the variables of interest. The northern basin was considered a suitable control site against which to compare the impact of the fish farm based on mass water movements. Drogue and remote sensing studies in Esthwaite Water have demonstrated that wind-induced currents are deflected by the Coriolis force (Falconer *et al.* 1991; George 1993). The resulting clockwise helical water flow in the epilimnion diminishes the strength of the flushing effect of the prevailing southerly winds. Thereby reducing transport by currents of phosphorus-rich waste from fish culture cages in the central impact basin to the northern control basin. It should also be noted that the general flow of water in Esthwaite Water is from the inflow at the north of the lake to the outflow at the south.

Sediment cores were collected at all 18 sampling sites using a 90 mm diameter gravity corer (Pylonex, Sweden). The top 4 cm of each core was extruded and frozen on the day of fieldwork for later analysis. At the deepest site in each basin, a peristaltic pump (Geopump, Geotech, Denver, USA) was used to collect water samples at 2 m intervals throughout the water column. Water samples were stored at 4 °C in 200 ml acid-washed, polycarbonate bottles prior to chemical analysis. At the same sites, depth profiles of pH and temperature were recorded on each sampling trip (Hydrolab DS5X Multi-Parameter Data Sonde, OTT Hydrometry, Sheffield, UK). A Plastimo Echotest II Depth Sounder (Southampton, UK) was used to measure water depth at each sampling point. Repeat sampling of the same locations each month was ensured by storing the site coordinates on a handheld GPS unit (eTrex 10, Garmin, Southampton, UK). Dissolved oxygen (DO) saturation data were obtained from the Cumbrian Lakes Long-Term Monitoring Scheme. Profiles are recorded fortnightly at the deepest point (16 m) in the northern control basin of Esthwaite Water (HQ30D Portable Meter, Hach, Salford, UK).

4.2.4 Water column total phosphorus analysis

Water column samples were stored at 4 °C between collection and TP analysis (within one week of collection). The potassium persulfate digestion method of Eisenreich *et al*. (1975) was used to oxidise all forms of phosphorus in the unfiltered samples to SRP. Digestion was followed by a modified version of the molybdenum blue method to determine TP concentration. In this procedure, SRP reacts with molybdate in an acidic medium to form molybdo-phosphoric acid, which is reduced by ascorbic acid to the intensely coloured phosphomolybdenum blue. The absorbance of this complex at 882 nm is used to quantify the concentration of phosphorus using a standard curve constructed from different concentrations of potassium dihydrogen orthophosphate (Murphy & Riley 1962). The limit of detection (LOD) for TP concentration was 5.0 µg l^{-1}. In each batch of samples analysed one sample was repeated in triplicate and four reference samples were included as analytical quality controls.

4.2.5 Sediment phosphorus analyses

In accordance with the work conducted on sediment samples retrieved from Esthwaite Water during fish farm operations (1986 to 2001, Section 4.2.2), sub-samples of all sediment samples collected throughout 2014 were analysed for AEP. The alkaline extraction reagent (0.125 M NaOH) was added to a 50 ml centrifuge tube containing 1 g of homogenised wet sediment. The sediment was exposed to the reagent for 16 h, during which time the mixture was continually shaken on a 360° rotator (Intelli-Mixer RM-2,

ELMI, Riga, Latvia). The supernatant was collected following centrifugation and the remaining sediment pellet exposed to fresh reagent for a further 5 min. The supernatant was collected as before and combined with the earlier supernatant to be filtered through a Whatman GF/C filter (pore size *c*. 1.2 µm) before TP analysis (Drake & Heaney 1987).

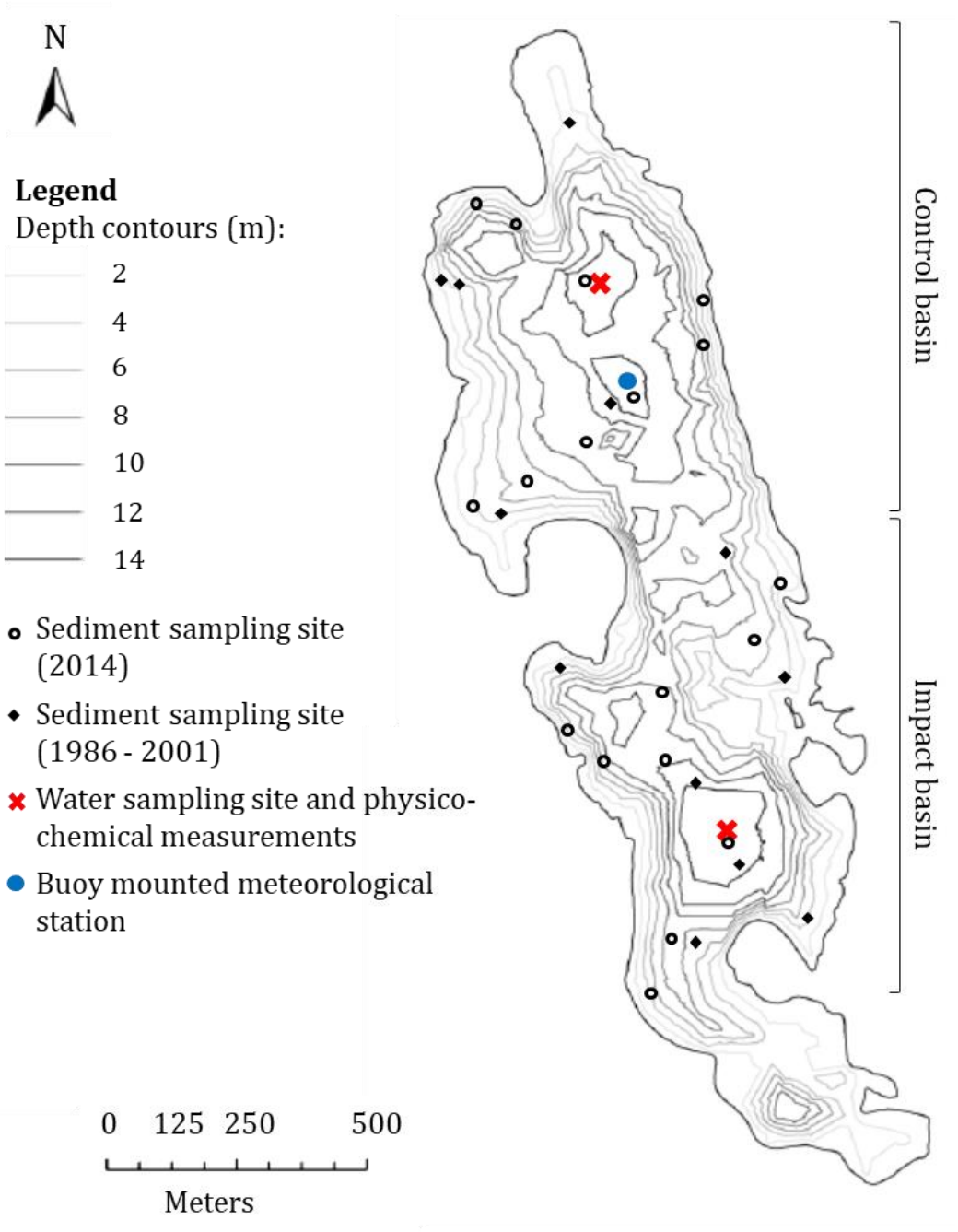

Figure 4.2: Bathymetric map of Esthwaite Water showing: Sediment sampling locations in the northern control and central impact basins, the locations of water column sampling and physico-chemical measurements in each basin, and the location of the buoy-mounted meteorological station. Adapted from Mackay *et al*. (2011).

Table 4.1: Sediment phosphorus extraction procedures. The exposure of sediment samples to reagents was conducted on an orbital shaker, unless stated otherwise.

Method	Sediment Phosphorus Fraction(s)		Reagent(s)	Volume	Exposure	Reference(s)
Alkali extraction (wet sediment)	AEP: (i) Labile; (ii) Reductant-soluble; and (iii) Organic		0.125 M NaOH	25 ml x 2	16 h followed by 5 min with fresh reagent, 25±1 °C	Drake & Heaney (1987)
Total extraction (dry sediment)	Sediment TP: All fractions		9.8 M H_2O_2 / 18 M H_2SO_4	4.4 ml	30 min at 220°C	Rowland & Grimshaw (1985)
			Deionised H_2O	45.6 ml	Vortexed briefly at high speed then left to stand until the sediment settled, at 25±1 °C	
Sequential extraction (wet sediment)	(i)	Labile	1 M NH_4Cl	25 ml	30 min at 25±1 °C	Psenner *et al*. (1988); Hupfer *et al*. (1995)
	(ii)	Reductant-soluble (e.g. bound to Fe-oxyhydroxides)	0.11 M $NaHCO_3$ / 0.11 M $Na_2S_2O_4$	25 ml x 2	1 h followed by 5 min with fresh reagent, at 25±1 °C	
	(iii)	a. Metal-oxide adsorbed b. Organic	1 M NaOH	25 ml x 2	16 h followed by 5 min with fresh reagent, at 25±1 °C	
	(iv)	Ca-bound (e.g. apatite)	0.5 M HCl	25 ml x 2	16 h followed by 5 min with fresh reagent, at 25±1 °C	
	(v)	Refractory	Deionised H_2O	40 ml	Vortexed at high speed for 1 min prior to TP analysis	

The total sediment phosphorus (sediment TP) concentration was also determined for all samples following the acid digestion method of Rowland and Grimshaw (1985). Sub-samples of sediment were air-dried and ground to a fine powder using a pestle and mortar; 0.35 g of dried sample were placed in a Pyrex Kjeldahl tube along with a hydrogen peroxide – sulphuric acid digestion mix and heated to 220 °C. When cool, the digest was diluted to 50 ml, mixed on a vortex mixer and the silica residue allowed to settle. Four reference samples and three blank solutions (digestion mixture only) were included per batch of samples digested. The TP concentration of the final solution was then measured by phosphomolybdenum blue reaction on a SEAL AQ2 Auto Analyser (Seal Analytical Ltd., Fareham, UK). The potassium dihydrogen orthophosphate standard solutions for the calibration curve were made-up using the diluted digest solution from the blank samples as the matrix. This was done to account for any bias that the digestion mixture might have contributed to the results.

Additionally, sub-samples of all sediment collected in January 2014 were subjected to phosphorus fractionation by the sequential extraction method of Hupfer *et al*. (1995) based on that of Psenner *et al*. (1988), detailed in Table 4.1. Extractions were conducted on 1 g of homogenised wet sediment. At each stage of the procedure, the sediment slurry and extraction reagent were shaken continually in 50 ml centrifuge tubes at room temperature on a 360° rotator. Supernatants were collected following centrifugation at the end of each extraction step and filtered through a Whatman GF/C filter (pore size *c*. 1.2 μm). All supernatants were analysed for TP and the supernatant from fraction (iii) underwent additional analysis for SRP [TP analysis without the initial persulfate digestion (Murphy & Riley 1962)]. The organic phosphorus fraction (iii b) was quantified by subtracting NaOH-SRP from NaOH-TP. The process was repeated three times using sub-samples from one of the sites (northern control basin, sample site 6) to determine variability within each step of the extraction scheme.

Sediment phosphorus concentrations are reported per unit dry weight sediment (μg g^{-1} DW). In addition to the sub-samples used for phosphorus analyses, three additional sub-samples (*c*. 1 g each) were collected from each sediment sample. These were weighed before and after drying at 105 °C for 48 h. The average dry weight (DW) per wet weight (WW) of a sediment sample was calculated by dividing the average DW of the three sub-samples by their average WW (g g^{-1}). The DW of the sub-sample used to determine phosphorus concentration was then calculated by multiplying the WW used (g) with the average DW per WW (g g^{-1}) of the sample. TP concentration (μg l^{-1}) was multiplied by the

volume of extraction reagent used (l) to give the mass of phosphorus (µg) in the sub-sample. Finally, phosphorus per g DW sediment (µg g^{-1}) was calculated by dividing the mass of phosphorus in the sub-sample with the average DW per WW.

4.2.6 Meteorological data

Wind speed and air temperature data were obtained from a buoy-mounted meteorological station in the centre of the northern control basin which takes measurements at four-minute resolution. The location of the buoy is shown in Figure 4.2. Hourly averages were used here.

4.2.7 Statistical analyses

Two different statistical analyses were conducted to assess the effect of fish farm operations on the sediment phosphorus content of Esthwaite Water. The first simply involved using Wilcoxon-Mann-Whitney non-parametric tests for control-impact and before-after comparisons of the monthly mean AEP concentrations of sediment collected during fish farm operations (1986 to 2001) and after the removal of culture cages (2014). The data were structured to allow for a like-with-like comparison between the before and after periods. As the historical sediment sampling locations did not correspond to the sites sampled in 2014, the monthly mean AEP concentrations of shallow (0 – 5 m), intermediate (5 – 10 m) and deep (> 10 m) sediment were calculated for each basin. A more complex Before-After-Control-Impact (BACI) approach was also applied. A BACI approach requires the comparison of an impacted site with a control site, both before and after the introduction of a potential stressor at the impact site (Stewart-Oaten *et al.* 1986). In this case, the stressor is the removal of the fish cages from the central impact basin of Esthwaite Water, so the analysis presented here is, in fact, a "reverse-BACI". The advantage of a BACI-type analysis, rather than simply comparing before and after data from an impacted site, is that natural seasonal and inter-annual variation can be distinguished from impact-induced variability. The same approach has been employed successfully in a previous study to determine the treatment effect of lake remediation measures (Lang *et al.* 2016).

Two-way Analysis of Variance (ANOVA) was used to test for a change in the difference between the mean sediment AEP concentration of the two basins in the periods before and after fish cage removal. The null hypothesis was that there was no change in the difference between before and after periods, i.e. no effect of fish cage removal on the sediment phosphorus content. Alternatively, detection of a significant difference (at the 5

% level) between the two time periods implied that the removal of fish cages did influence the sediment AEP concentration of the central impact basin. In addition to testing the effect of site (basin) and year, the ANOVA also included a year-site interaction to determine if the difference in sediment AEP concentrations between basins was dependent on the year. Historical AEP data from the years 1986, 1992 and 2001 were selected for comparison against the data collected in 2014. Although monthly sediment AEP data were available for 2014, sediment sampling between 1986 and 2001 had been conducted with less regularity. The disparity in temporal resolution between the periods was addressed by only using the AEP mean values for months from 2014 for which there was corresponding monthly data from the year used for the before period. Again, AEP concentrations were averaged by depth zone.

General linear models were used to investigate the spatial variability in contemporary sediment TP concentration and AEP concentration. Basin (northern control or central impact), water depth, sampling month and the interaction between the three were included as explanatory variables. In addition, the model was refitted with second-order polynomial depth terms to allow for potentially non-linear changes of phosphorus with depth. Model Akaike information criterion (AIC) values were compared to select the optimal combination of explanatory variables for inclusion in the final model. Prior to model fitting, sediment TP and AEP concentrations were log-transformed to satisfy parametric test assumptions. For each fraction of the sequential extraction procedure, Wilcoxon-Mann-Whitney tests were used to compare the mean phosphorus concentrations between the northern control basin and central impact basin.

Sediment samples collected in January 2014 were the only samples for which sediment phosphorus concentration was analysed using all three extraction methods: sequential extraction, alkali extraction (NaOH exposure) and sediment TP extraction (H_2O_2 – H_2SO_4 digestion). To assess the reliability and effectiveness of the different methods, pairwise comparisons were made between the results from different extraction techniques. Sediment TP concentration was compared to the sum of all sediment phosphorus fraction concentrations determined by the sequential extraction procedure. AEP was compared to the sum of labile, reductant-soluble and organic phosphorus concentrations as determined by the sequential extraction procedure. Paired Wilcoxon-Mann-Whitney tests were used to determine whether the phosphorus concentrations measured by single-step extraction and sequential extraction were significantly different.

Pearson product-moment correlations were used to assess the linear relationship between sediment AEP concentration or water column TP concentration and the potential drivers of sediment phosphorus mobility. Potential drivers included water temperature, DO concentration, pH, air temperature and the cube of wind speed. Studies in other lakes reveal strong vertical zonation in the processes that drive sediment phosphorus release (Spears *et al.* 2007b, 2012). To account for this zonation of processes, monthly water column temperature profiles were used to identify the typical thermocline depth during lake stratification. The thermocline between May and September generally fluctuated within the 5 m to 10 m depth zone. Therefore, sampling sites were divided into three depth categories; epilimnion (< 5 m depth), metalimnion (5 m – 10 m depth) and hypolimnion (> 10 m depth). Monthly volume-weighted averages of TP concentration, DO saturation, pH and temperature were calculated for the three depth zones of both the control and central impact basin using a bathymetry for the lake (Mackay *et al.* 2012). When TP was below the analytical LOD a value of 2.5 μg l^{-1} (half of the 5.0 μg l^{-1} LOD) was used in calculations of the water column mean TP concentration. Monthly volume-weighted means of sediment phosphorus concentration (sediment TP and AEP) were calculated for shallow sediments (collected at < 5 m water depth), intermediate sediment (collected between 5 m and 10 m) and deep sediments (collected at depths > 10 m).

Daily mean air temperature and wind speed data were also averaged by month. Air temperature is a proxy for water column thermal structure and associated physico-chemical variability (Heaney *et al.* 1986; Maberly 2008; Mortimer 1941; Robertson & Ragotzkie 1990). It was included to substantiate evidence for the regulation of sediment phosphorus mobility by pH, DO concentration and water temperature. Disruption of the sediment surface by wind-induced mixing is a potential driver of sediment phosphorus release (Hamilton & Mitchell 1997; Søndergaard *et al.* 1992). The rate at which energy is imparted to the water column is proportional to the cube of wind speed cubed (Fischer *et al.* 1979). For that reason, wind speed values were cubed to obtain a proxy of wave-mixed depth. To reduce the likelihood of obtaining a significant correlation between the potential driving variables and response variables due to their shared seasonality rather than cause-effect relationship, correlations were conducted using the residuals of polynomial regression fits to time series data to remove their seasonal component. All statistical analyses were carried out using R statistical software package version 3.2.3 for Windows (R Core Team 2016).

4.3 Results

4.3.1 Water column physico-chemical conditions

Water column profiles of temperature and pH displayed similar seasonal patterns at the northern control basin and the central impact basin. Between January and March the water column was well mixed, by May a strong thermocline was established between 6 m and 8 m depth in both basins which persisted until September (Figure 4.3a/c). In June, the epilimnion mean temperature of the central impact basin reached an annual maximum of 20.5 °C compared to a hypolimnion mean temperature of 12.3 °C. A similar difference in epilimnion and hypolimnion temperature was recorded in the northern control basin. In both basins, hypolimnion mean pH remained relatively constant throughout the year whereas the epilimnion mean pH increased during the period of thermal stratification (Figure 4.3b/d).

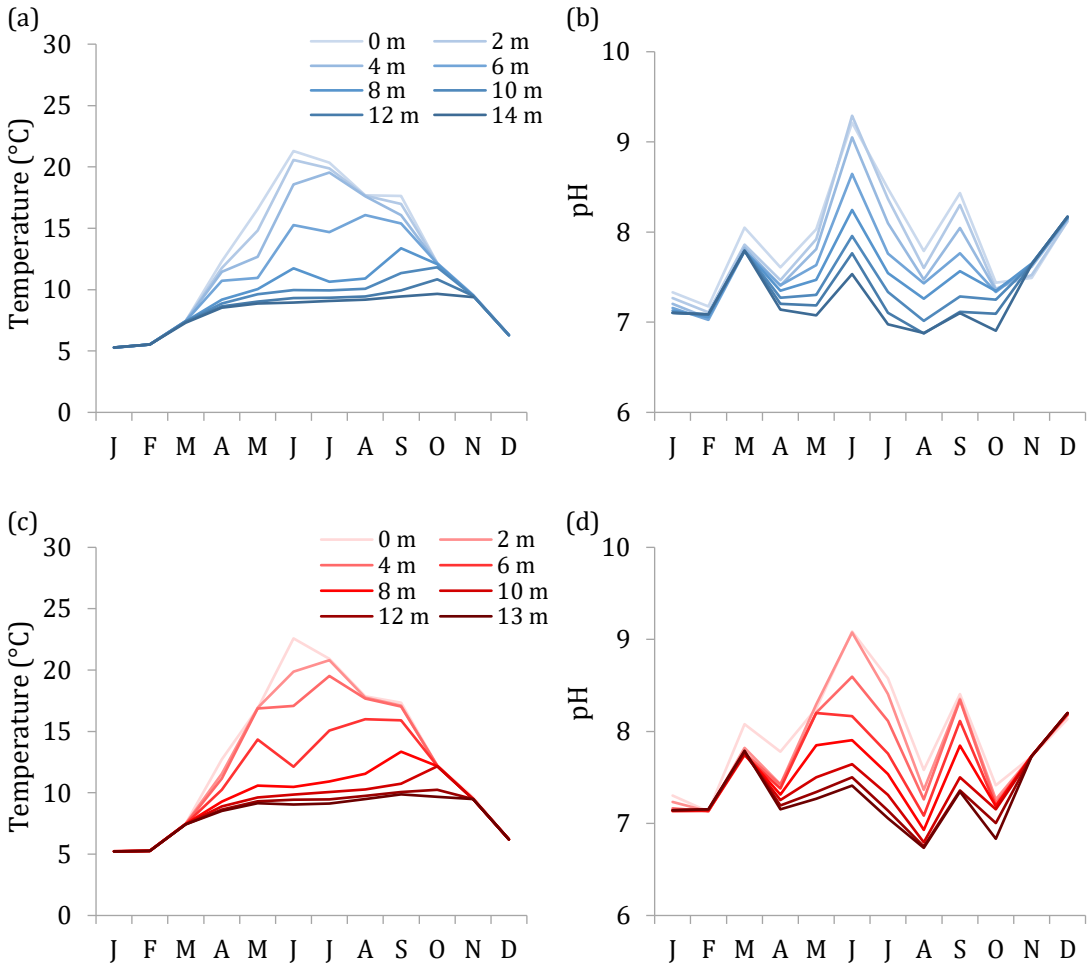

Figure 4.3: Water column profiles of physico-chemical variables for Esthwaite Water, conducted monthly between January and December 2014: Northern control site water temperature (a) and pH (b), central impact site water temperature (c) and pH (d).

Monthly mean DO saturation profiles collected in the northern control basin for the Cumbrian Lakes Long-Term Monitoring Scheme also exhibit a strong seasonal pattern (Figure 4.4). In the first three months and last three months of 2014 the whole water column was well oxygenated. Between April and September two distinct depth-zones developed; the surface 5 m of the water column remained well oxygenated while the deeper water became progressively more depleted in oxygen. Between July and September, the bottom 7 m of the lake was anoxic.

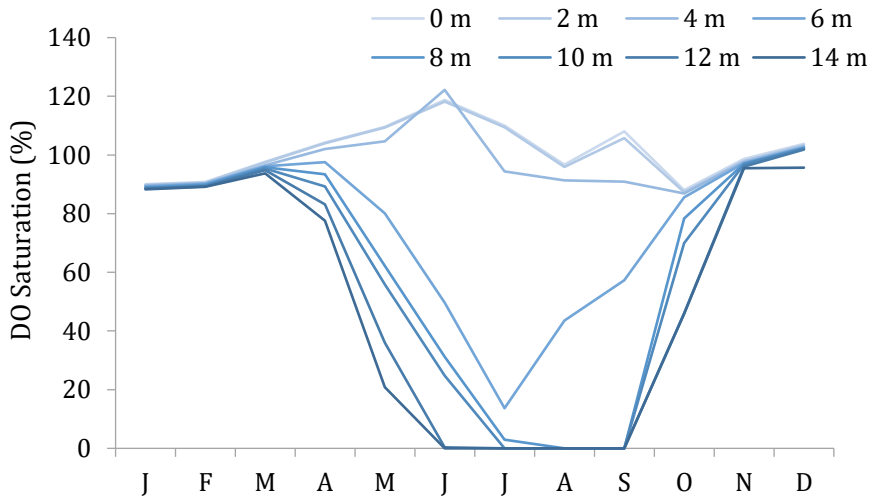

Figure 4.4: Monthly mean dissolved oxygen saturation depth profile for Esthwaite Water between January and December 2014. Measurements were taken as part of the Cumbrian Lakes Long-Term Monitoring Scheme as at the same location as the northern control site measurements of other physico-chemical variables.

4.3.2 Water column total phosphorus concentration

During the first few months of the year, there was little difference between the mean TP concentrations of the three depth zones in either the northern control basin or central impact basin (Figure 4.5). From April through to October the hypolimnion mean TP concentration in the northern control basin was markedly higher than the epilimnion and metalimnion mean TP concentrations. In the central impact basin, hypolimnion mean TP concentration also surpassed the epilimnion and metalimnion means but not until August. At both sites the hypolimnion mean TP concentration peaked in September at *c.* 190 µg l^{-1}. From November to the end of the year the TP concentration in both basins was homogenous throughout the water column. The epilimnion and metalimnion mean TP concentrations in both basins remained relatively stable, between 10 µg l^{-1} and 30 µg l^{-1},

throughout the year. In the central impact basin, metalimnion mean TP concentration spiked in September to *c.* 75 µg l^{-1}. Annual mean epilimnion concentrations, calculated as the average of monthly means, were 22.3 µg l^{-1} in the northern control basin and 20.5 µg l^{-1} in the central impact basin.

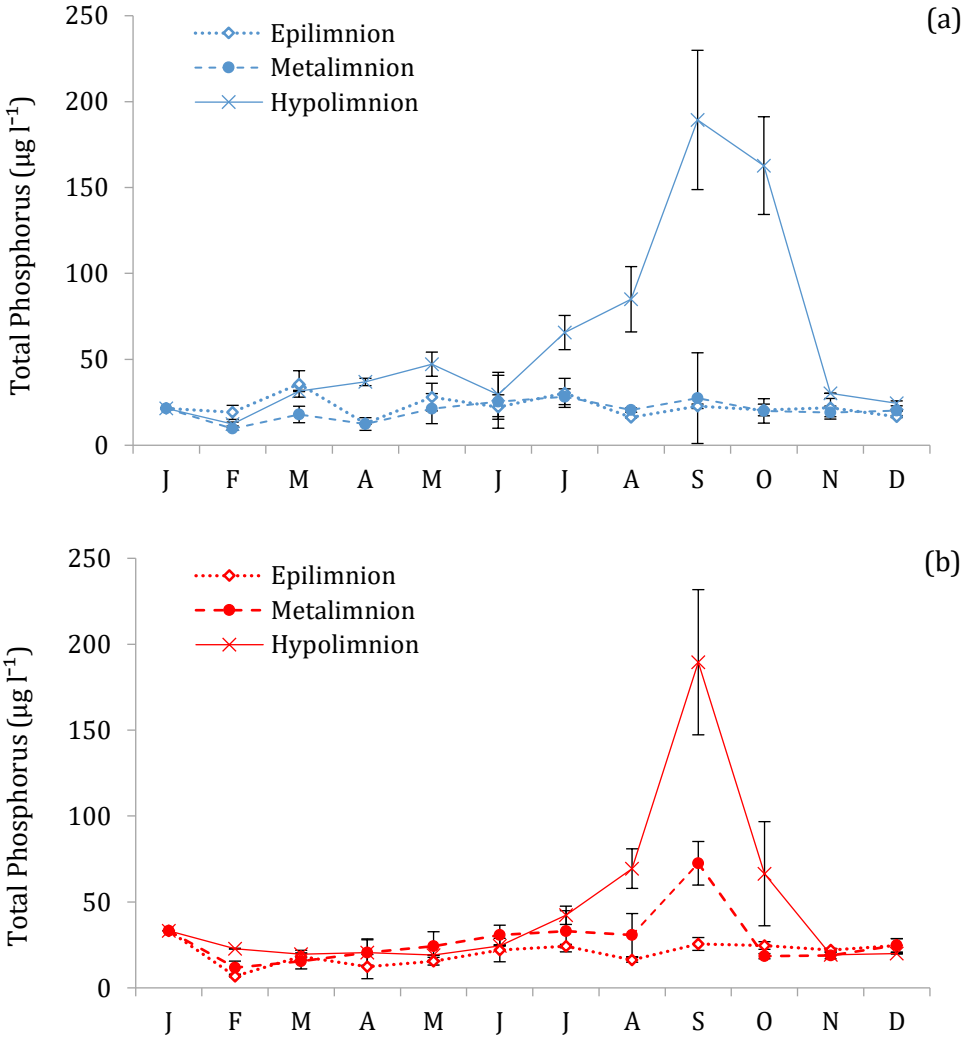

Figure 4.5: Monthly volume-weighted mean total phosphorus concentration of the epilimnion (< 5 m), metalimnion (5 – 10 m), and hypolimnion (> 10 m) throughout 2014: (a) Northern control site, and (b) central impact site. Error bars represent the standard deviation of the volume-weighted mean.

4.3.3 Comparison of historical and contemporary AEP concentrations

Results of the impact-control comparison using Wilcoxon-Mann-Whitney tests suggested that the fish farm significantly enhanced the sediment AEP concentration of the central impact basin during its operation (impact mean = 2388 µg g^{-1} DW, control mean = 1465 µg g^{-1} DW, $p = 0.04$). The before-after comparison of the contemporary sediment AEP data

to the historical data showed that the overall mean concentration is significantly higher now than it was before the aquaculture cages were removed (Table 4.2). Comparison of individual basins revealed no significant difference between the historical and contemporary mean AEP concentration of the central impact basin sediments but a significantly higher concentration in the northern control basin at present than during fish farm operations.

Table 4.2: Summary of Wilcoxon-Mann-Whitney tests to compare the historical (1986 to 2001) and contemporary (2014) AEP concentrations of surface sediment. Monthly means of AEP concentration were calculated for the specified basin and depth zone. Bold, underscored text denotes a significant difference ($p < 0.05$).

Basin	Depth Zone(s)	AEP (μg g^{-1} DW)		*p*
		Historical	Contemporary	
Whole lake	All	1927	2793	**<0.001**
Northern control	All	1465	3003	**<0.001**
Central impact	All	2388	2558	0.44
Northern control	Shallow	1021	1442	**<0.05**
Northern control	Intermediate	1489	2231	**<0.001**
Northern control	Deep	2752	5278	**<0.001**
Central impact	Shallow	1134	1317	0.24
Central impact	Intermediate	1613	2027	0.15
Central impact	Deep	4658	4130	0.30

Table 4.3: Results of the two-way ANOVAs to compare AEP concentrations of sediments from the central impact basin and northern control basin before (1986, 1992 or 2001) and after (2014) the removal of the aquaculture cages, using a BACI design. Bold, underscored text denotes a significant difference ($p < 0.05$).

Before Year	Explanatory Variable	d.f.	Sum Sq.	Mean Sq.	*F*	*p*
1986	Year	1	1.43	1.43	4.06	**<0.05**
	Basin	1	0.04	0.04	0.11	0.74
	Year-basin interaction	1	0.95	0.95	2.66	0.10
1992	Year	1	1.63	1.63	5.48	**0.02**
	Basin	1	0.01	0.01	0.04	0.84
	Year-basin interaction	1	1.07	1.07	3.58	0.06
2001	Year	1	0.50	0.50	1.38	0.25
	basin	1	0.01	0.01	0.03	0.87
	Year-basin interaction	1	0.21	0.21	0.57	0.46

The outputs of the ANOVA tests confirm a significant difference between the mean AEP concentrations of the before and after periods due to temporal effects unrelated to the impact, in two of the three ANOVA tests [1986 (p = 0.049); 1992 (p = 0.02)]. However, the ANOVA tests did not determine a significant difference in the overall mean AEP concentration between the central impact and northern control basins (Table 4.3). The BACI effect was represented in the ANOVA test by the period-basin interaction. It was not determined to have a significant effect on sediment AEP concentration, which implies that removal of the fish cages from the central impact basin did not result in a significant change in the difference between the mean AEP concentrations of the two basins. Although the ANOVA tests did not confirm that the fish farm operations and the subsequent removal of cages had an environmental impact on the sediments in Esthwaite Water, the profile plots show non-parallelism of the response between the northern control and the central impact basin (Figure 4.6). Whereas the mean AEP concentration of the central impact basin remained relatively unchanged between the before and after period, there was a noticeable increase in the northern control basin since sediment was last sampled during fish farm operations in 2001. The increased AEP concentration of sediments from the northern control basin is likely accountable for the significant difference determined between the overall mean of the before and after periods.

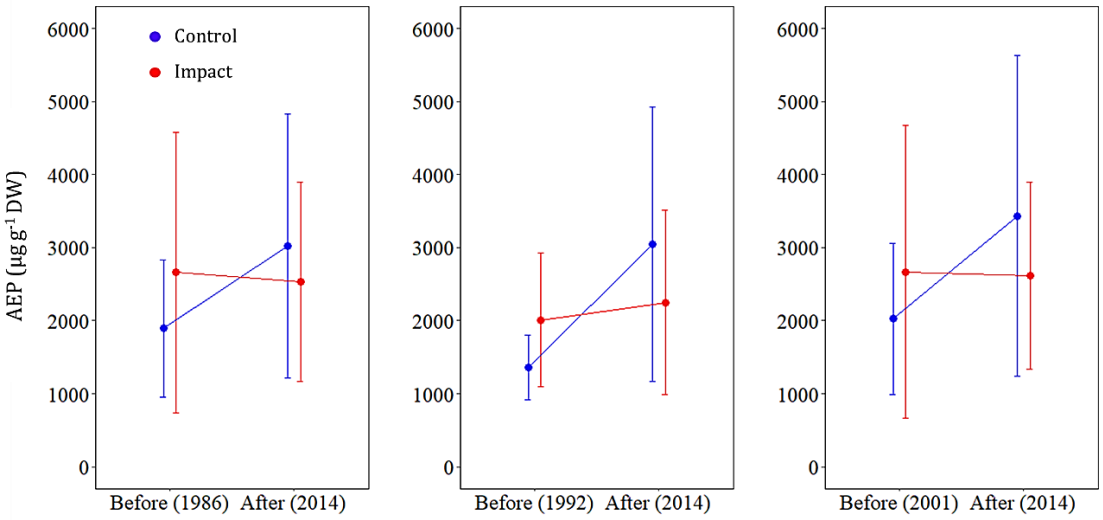

Figure 4.6: Mean AEP concentration of sediments from the northern control basin and central impact basin, before and after aquaculture cage removal: 1986 compared to 2014 (n = 30), 1992 compared to 2014 (n = 12), and 2001 versus 2014 (n = 6). Error bars represent standard deviation of the mean.

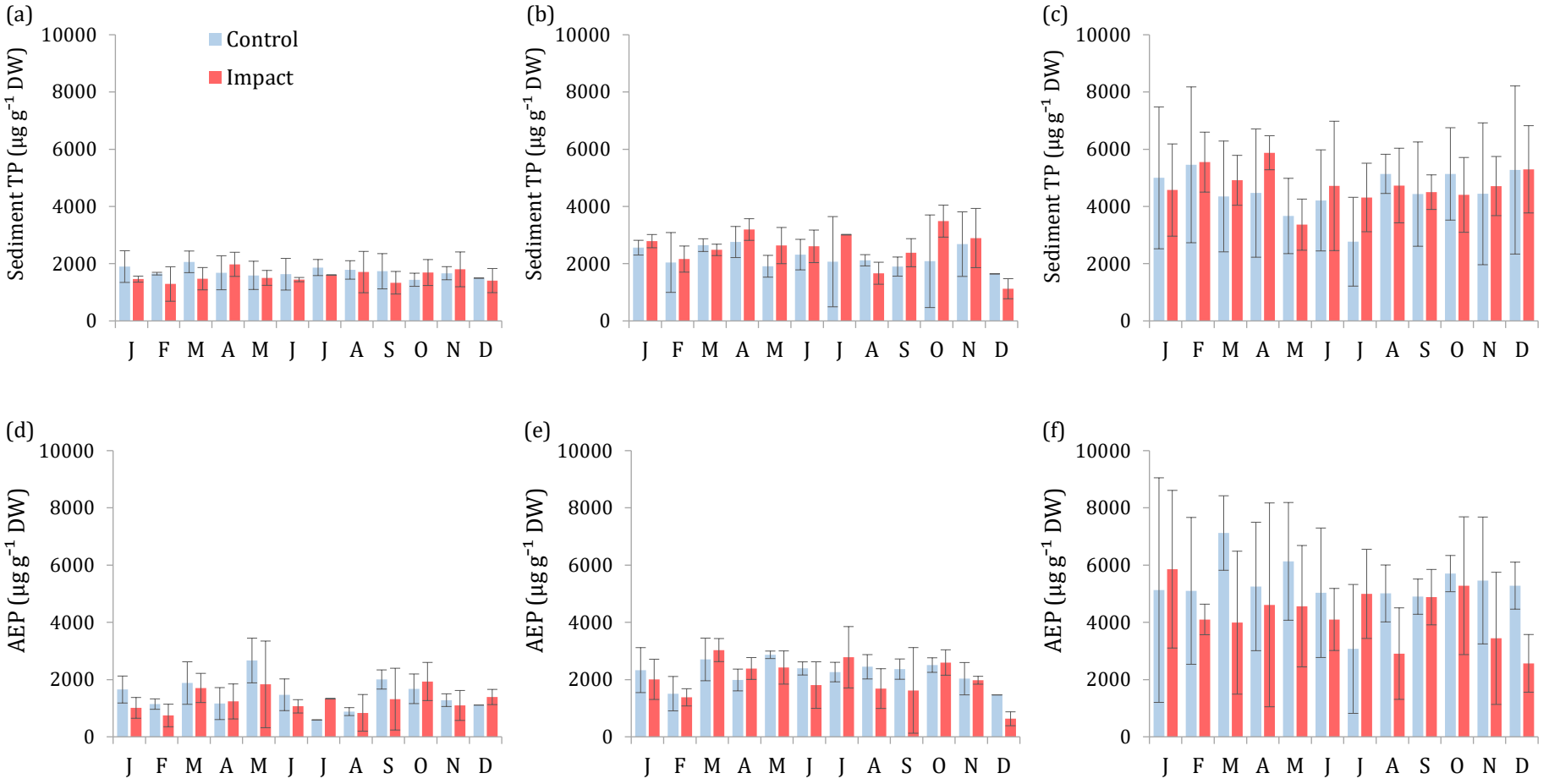

Figure 4.7: Monthly means of sediment TP concentration of surface sediment in the (a) shallow depth zone, (b) intermediate depth zone, and (c) deep depth zone, and AEP concentration of surface sediment in the (d) shallow depth zone, (e) intermediate depth zone, and (f) deep depth zone. The error bars represent standard deviation of the mean (n = 3).

4.3.4 Spatial and temporal variation in contemporary sediment phosphorus concentrations

Mean concentrations of sediment TP and AEP by month, basin and depth zone are shown in Figure 4.7. There is no obvious seasonality to either total phosphorus concentration (Figure 4.7a-c) or AEP concentration (Figure 4.7d-f). It is, however, clear from these graphs that the concentration of each increases from the shallow zone to the deep zone. General linear models with basin, depth and month as their explanatory variables were used to investigate the spatial and temporal variation in sediment phosphorus concentrations. Comparison of linear model AIC values revealed that month was not an important explanatory variable and that no additional variance could be significantly explained by using a polynomial rather than a linear fit to test the effect of depth on sediment phosphorus concentrations. The single best model for both sediment TP and AEP included basin, depth and basin-depth interaction. Basin and overlying water depth were both found to have a significant effect on sediment-bound phosphorus variation in Esthwaite Water (Table 4.4).

Table 4.4: Results of the best-fit general linear models used to investigate spatial variation in sediment phosphorus concentration. Statistically significant results are denoted by bold, underscored text ($p < 0.05$).

Response Variable	Explanatory Variable	Est.	Std. Err.	*t*	*p*
Sediment TP	Intercept (central impact basin)	7.14	0.05	129.87	<0.001
	Basin (northern control basin)	-0.19	0.08	-2.30	**0.02**
	Depth	0.09	0.01	14.54	**<0.001**
	Basin-depth interaction	0.03	0.01	3.50	**<0.001**
AEP	Intercept (central impact basin)	6.86	0.08	85.28	<0.001
	Basin (northern control basin)	0.15	0.12	1.26	0.21
	Depth	0.12	0.01	13.48	**<0.001**
	Basin-depth interaction	0.01	0.01	0.49	0.62

The mean sediment TP concentration of the central impact basin (3034 μg g^{-1} DW) was significantly higher than that of the northern control basin (2851 μg g^{-1} DW, $p = 0.02$). Basin-depth interaction also had a significant effect on sediment TP ($p < 0.001$). The concentration increased with increasing water depth in both basins, but the relationship was significantly stronger in the central impact basin than in the northern control basin (Figure 4.8a). In contrast to sediment TP, the northern control basin AEP mean (3032 μg g^{-1} DW) was higher than that of the central impact basin (2628 μg g^{-1} DW). However, the

difference was not determined to be significant (p = 0.21). There was a significant depth effect on AEP (p < 0.001). In both basins, the AEP concentration of the sediment increased with increasing water depth but there was not a significant difference in the strength of this relationship between basins (Figure 4.8b).

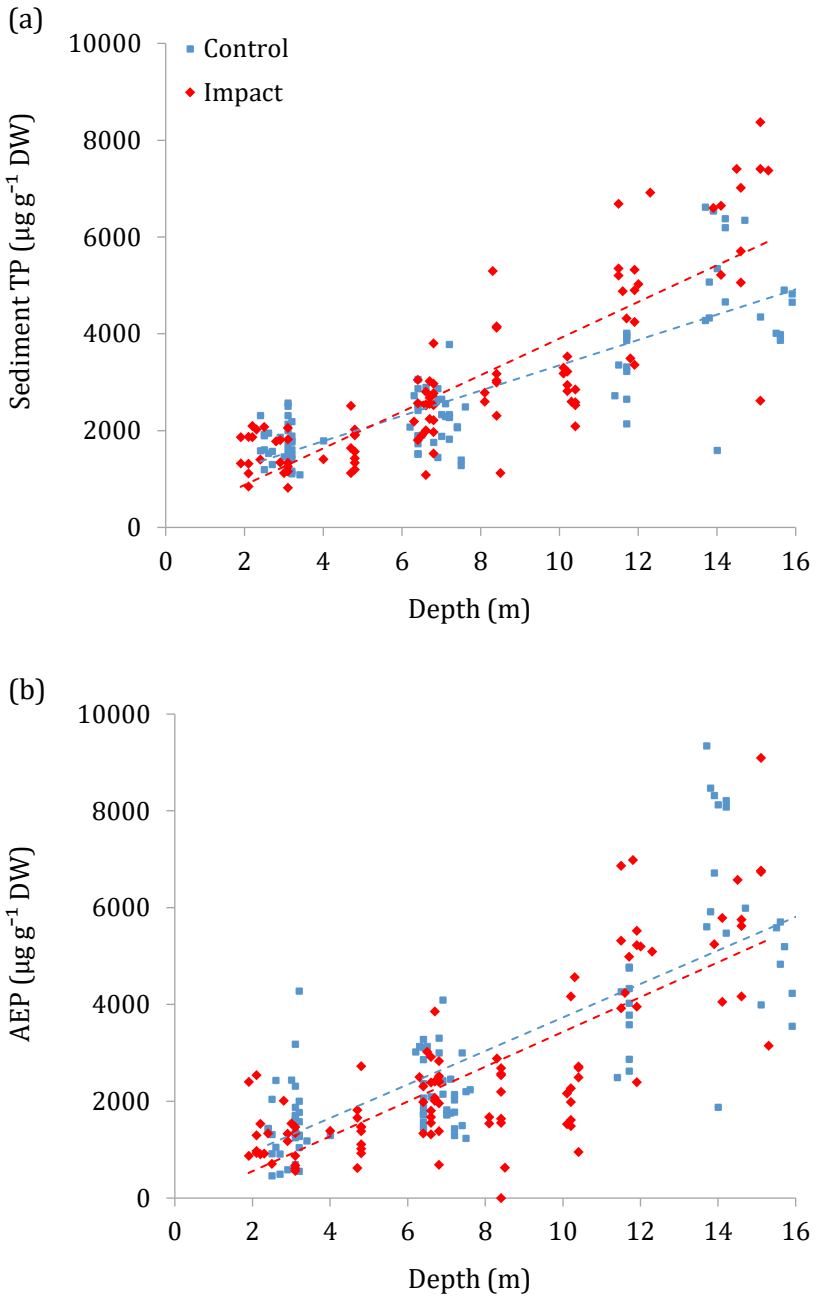

Figure 4.8: Variation with water depth of (a) sediment TP concentration and (b) AEP concentration of surface sediments sampled from Esthwaite Water in 2014.

4.3.5 Sediment phosphorus fractionation

The error within each step of the extraction scheme was ±61 % for the labile fraction, ±17 % for the reductant-soluble fraction, ±25 % for the metal-adsorbed fraction, ±43 % for the organic fraction, ±19 % for the apatite-bound fraction, and ±11 % for the refractory fraction. Reductant-soluble phosphorus comprised the largest portion of sediment-bound phosphorus in shallow, intermediate and deep sediments from both the central impact basin and northern control basin (Figure 4.9). The concentration of labile phosphorus was low at all sites (< 1 % of the sum of fractions). In both basins, the deep sediments tended to be more enriched with all phosphorus fractions than shallow or intermediate sediments. The mean concentration of each phosphorus fraction in deep sediment was consistently greater in the central impact basin than in the northern control basin but the same pattern was not observed for sediments in either the shallow or intermediate depth zone. A significant difference between the central impact basin and northern control basin was only found for the apatite-bound phosphorus fraction ($p < 0.01$).

4.3.6 Comparison of phosphorus extraction techniques

Pairwise comparisons were made between the phosphorus concentrations obtained using different extraction techniques on sub-samples from the same sample. For the 18 sediment samples collected in January 2014 AEP concentration was subtracted from the sum of labile, reductant-soluble and organic phosphorus (mobile phosphorus) concentrations. Likewise, the sediment TP concentration was subtracted from the sum concentration of all sediment phosphorus fractions. AEP concentration determined by single-step alkali extraction tended to be higher than the summed concentration of sequentially extracted mobile phosphorus fractions (Figure 4.10). The difference, as determined by paired Wilcoxon-Mann-Whitney tests, was significant ($p = 0.04$). The mean AEP concentration of samples subjected to the single-step alkali extraction was 2998 μg g^{-1} DW compared to the mean concentration of 2310 μg g^{-1} DW calculated from the sum of sequentially extracted mobile phosphorus fractions for each sample. Sediment TP concentration was significantly lower ($p < 0.01$) in samples analysed by hydrogen peroxide – sulphuric acid digestion (mean = 3047 μg g^{-1} DW) than samples for which sediment TP was measured by the sequential extraction procedure (mean = 3877 μg g^{-1} DW).

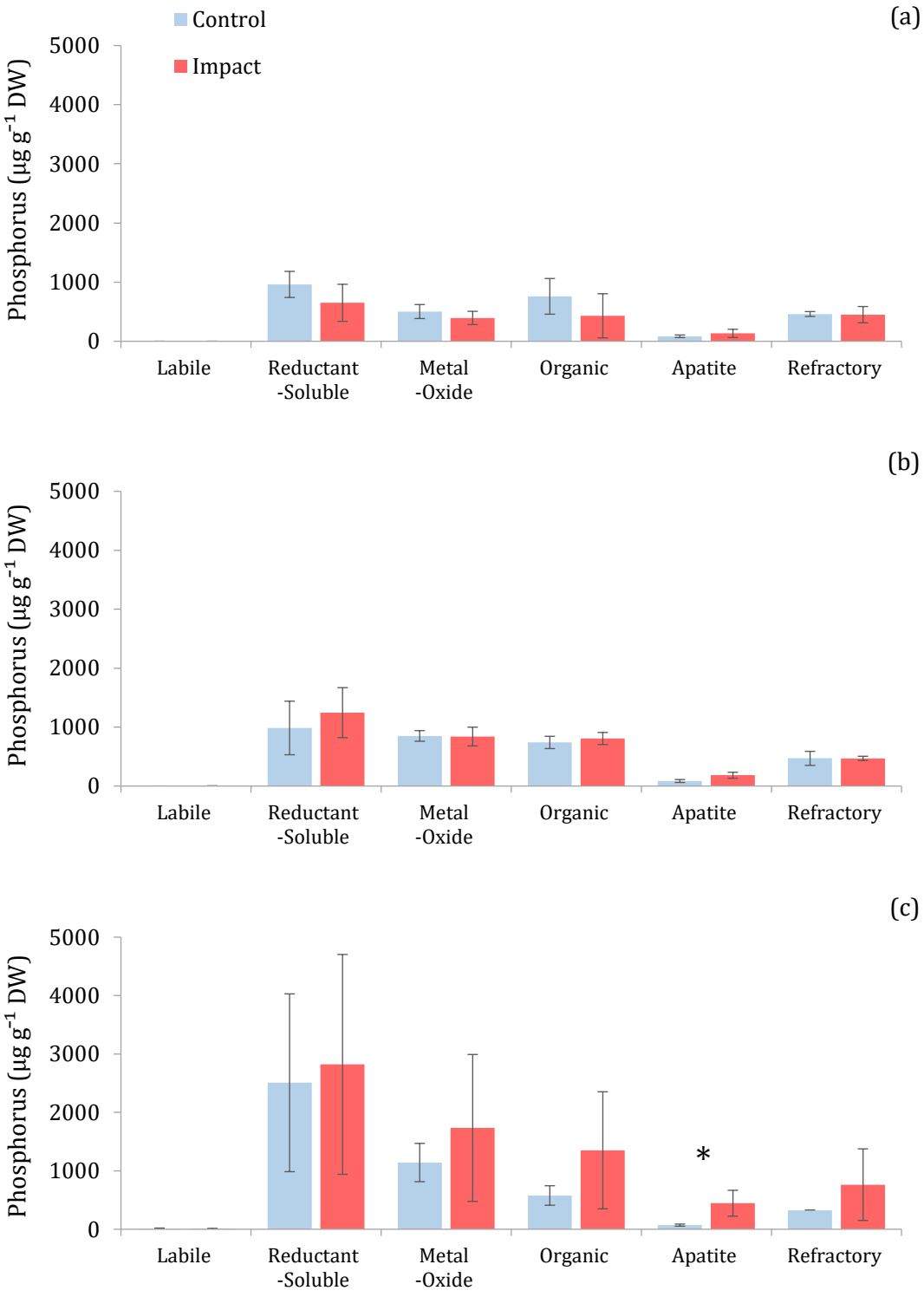

Figure 4.9: Mean concentrations of phosphorus fractions sequentially extracted from sediments of the northern control basin and the central impact basin: (a) shallow sediment, < 5 m water depth; (b) intermediate sediment, 5 – 10 m depth; and (c) deep sediment, > 10 m depth. The error bars represent standard deviation of the mean (n = 3). * denotes a significant difference between basins ($p < 0.05$).

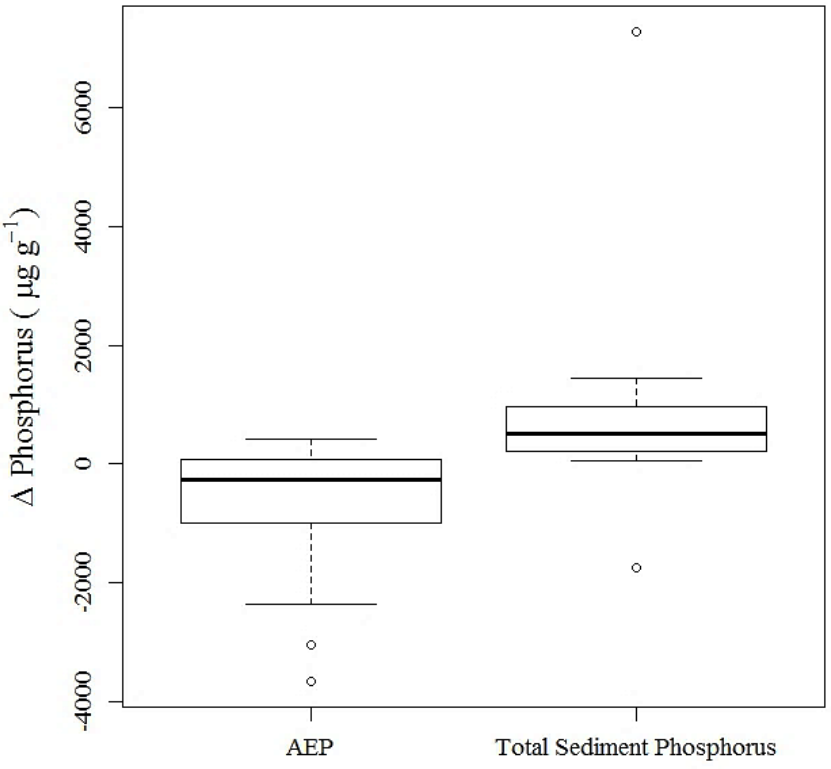

Figure 4.10: Box and whisker plots of the difference between sediment phosphorus concentrations determined by single-step extraction and by sequential extraction of phosphorus fractions. For 18 sediment samples collected in January 2014 AEP concentration was subtracted from the sum of labile, reductant-soluble and organic phosphorus concentrations, and the sediment TP concentration was subtracted from sum of concentrations for all phosphorus fractions. The black line indicates the median, the 25th and 75th percentiles are represented by the box, whiskers show the 10 – 90 % percentiles and outliers are represented as circles.

The percentage contribution of AEP to sediment TP was considerably higher when based on the results of the single-step extraction procedures than the results of the sequential extraction procedure. On average, AEP accounted for 90 % of sediment TP. When contributions were assessed by site, AEP equated to 92 % of the sediment TP in the northern control basin and 88 % in the central impact basin. Based on the results of the sequential extraction procedure, AEP accounted for 58 % of sediment TP on average when samples from both basins were taken into account, or 61 % in the northern control basin and 55 % in the central impact basin.

4.3.7 Potential mechanisms of sediment phosphorus mobility

A series of Pearson product-moment correlations were conducted to investigate associations between sediment AEP concentration and environmental conditions, to give an indication of the possible mechanisms or processes regulating phosphorus mobility across the sediment-water interface. Prior to analysis seasonality was removed from the variables, therefore the likelihood of determining a significant relationship due to shared seasonality of variables rather than cause-effect was reduced. Firstly, the relationships between monthly mean concentrations of AEP and water column TP were tested. No significant correlations were determined for either the northern control or central impact basin (Table 4.5). However, analysis of sediment and water column phosphorus concentrations with meteorological and physico-chemical variables did produce some significant results. There was a strong negative correlation between deep sediment AEP concentration and air temperature in the northern control basin ($p < 0.01$, $r = -0.75$), and a positive correlation between deep sediment AEP concentration and water temperature in the central impact basin ($p = 0.04$, $r = 0.59$).

Epilimnion TP concentration in the northern control basin was not significantly correlated to any of the other measured variables. On the other hand, epilimnion TP concentration of the central impact basin was significantly correlated to cube of wind speed ($p = 0.02$, $r = -0.67$), air temperature ($p < 0.01$, $r = 0.75$), and lake temperature ($p = 0.01$, $r = 0.70$). In both basins, metalimnion TP concentration was positively correlated with air temperature (control $p < 0.01$, $r = 0.78$; impact $p = 0.05$, $r = 0.58$), and negatively correlated with DO saturation (control $p = 0.02$, $r = -0.67$; impact $p = 0.06$, $r = -0.63$). In the central impact basin, there was also a positive relationship between the metalimnion temperature and TP concentration ($p = 0.04$, $r = -0.60$). In the northern control basin, hypolimnion TP concentration correlated negatively with pH ($p = 0.04$, $r = -0.59$). The hypolimnion TP concentration of the central impact basin was not significantly correlated to any of the physico-chemical variables, although the relationship with hypolimnion DO saturation was close to significant ($p = 0.06$, $r = -0.55$).

Table 4.5: Summary of Pearson product-moment correlations between sediment AEP concentration, water column TP concentration, meteorological variables and water column physico-chemical variables. Monthly mean values were used (n = 12). Bold, underscored text denotes a significant correlation ($p < 0.05$).

Variable	Sediment AEP						Water Column TP					
	Shallow		Intermediate		Deep		Epilimnion		Metalimnion		Hypolimnion	
	p	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*
Northern Control Basin												
Water column TP	0.29	0.34	0.14	0.45	0.94	0.02	-	-	-	-	-	-
Cube of wind speed	0.44	-0.24	0.10	-0.50	0.86	-0.06	0.64	-0.15	0.07	-0.54	0.76	0.10
Air temperature	0.40	-0.27	0.83	0.07	**<0.01**	**-0.75**	0.79	0.09	**<0.01**	**0.78**	0.28	0.34
Lake temperature	0.48	-0.23	0.34	0.30	0.38	0.28	0.77	-0.10	0.12	0.47	0.14	0.45
pH	0.82	0.07	0.99	0.00	0.23	0.38	0.34	0.30	0.36	0.29	**0.04**	**-0.59**
DO saturation	0.78	0.09	0.85	0.06	0.07	0.53	0.85	0.06	**0.02**	**-0.67**	0.16	-0.43
Central Impact Basin												
Water column TP	0.54	0.19	0.33	-0.31	0.17	0.43	-	-	-	-	-	-
Cube of wind speed	0.58	-0.18	0.36	-0.29	0.45	-0.24	**0.02**	**-0.67**	0.26	-0.35	0.98	-0.01
Air temperature	0.38	-0.28	0.88	-0.05	0.05	0.57	**<0.01**	**0.75**	**0.05**	**0.58**	0.17	0.42
Lake temperature	0.33	-0.31	0.54	0.20	**0.04**	**0.59**	**0.01**	**0.70**	**0.04**	**0.60**	0.51	0.21
pH	0.74	0.11	0.79	-0.09	0.23	-0.38	0.24	0.36	0.66	0.14	0.33	-0.31
DO saturation	0.80	-0.08	0.27	0.35	0.30	-0.33	0.71	0.12	**0.03**	**-0.63**	0.06	-0.55

Inter-relationships between the potential drivers of sediment phosphorus mobility were also tested (Table 4.6). Strong significant relationships were determined between air temperature and the mean temperature of the epilimnion ($p < 0.001$, r = 0.85) and the metalimnion ($p < 0.01$, $r = 0.57$), and between air temperature and the mean DO concentration of the metalimnion ($p < 0.001$, $r = -0.86$) and hypolimnion ($p < 0.001$, $r = -0.85$).

Table 4.6: Summary of the Pearson product-moment correlations between meteorological and physico-chemical variables. Monthly mean values were used (n = 12). Bold, underscored text denotes a significant correlation ($p < 0.05$).

Variables		Epilimnion		Metalimnion		Hypolimnion	
Meteorological	Physico-chemical	*p*	*r*	*p*	*r*	*p*	*r*
Cube of wind speed	Lake temperature	0.08	-0.36	0.21	-0.26	0.14	-0.31
Cube of wind speed	pH	0.08	-0.36	0.12	-0.33	0.23	-0.25
Cube of wind speed	DO saturation	0.06	-0.39	0.94	0.02	0.93	0.02
Air temperature	Lake temperature	**<0.001**	**0.85**	**<0.01**	**0.57**	0.80	0.05
Air temperature	pH	0.28	0.23	0.48	-0.15	0.06	-0.38
Air temperature	DO saturation	0.91	-0.02	**<0.001**	**-0.86**	**<0.001**	**-0.85**

4.4 Discussion

4.4.1 Impact of aquaculture on the spatial distribution of sediment phosphorus

Analysis of historical data collected during fish farm operations in Esthwaite Water indicates that the presence of salmonid culture cages in the central impact basin had the effect of enhancing sediment AEP concentration relative to the northern control basin. Unfortunately, data were not available from the period prior to the installation of the fish cages against which to compare the data collected during their operation. As such, it is impossible to conclude that this enrichment was the result of aquacultural practices. However, given that the two basins were likely subjected to very similar environmental conditions otherwise, and the main input from WwTWs is to the northern control basin, it is logical to assume that the difference in mean sediment AEP concentration was due to the discharge of phosphorus-rich waste from the culture cages to the central impact basin.

A simple before-after comparison suggests that the removal of fish cages from the lake in 2009, and the consequent cessation of waste inputs, had little effect on the AEP concentration of sediments from the central impact basin. However, the interpretation of this result is confounded when the trajectory of sediment AEP concentration in the northern control basin is also considered. While the BACI analysis confirmed that there was not a significant change in mean sediment AEP concentration in the central impact basin since the removal of the fish farm, it did determine a significant increase in the overall mean AEP concentration of lake sediments between 2001 and 2014. The mean AEP concentration of sediment in the northern control basin approximately doubled during this period. There are a few possible explanations for the increase, for example, an increase in the external phosphorus load to the lake or changes in the biogeochemical composition of the sediment, which resulted in the increased uptake of phosphorus from the water column. One of the fundamental assumptions of BACI analysis is that the environmental conditions which influence long-term trends in the response variable are comparable between the control and impact site (Stewart-Oaten *et al.* 1986). If variations in external phosphorus load or sediment characteristics were localised to the northern basin, its selection as control site in a BACI analysis to investigate the impact of the fish cage removal on the central impact basin would not be appropriate. Alternatively, it is possible that removal of aquaculture cages would have led to a decrease in sediment AEP concentration in the central impact basin, but this effect was masked by a lake-wide increase in sediment phosphorus uptake in recent years due to factors unrelated to fish farm operations. Another explanation is that the northern control basin, as the discharge point for most of the inflows to Esthwaite Water, retains much of the external phosphorus load, effectively buffering the central impact basin from increases in the external load over time. Therefore, the impact of phosphorus-rich aquaculture waste on sediment was not as obvious as it might have been had the external phosphorus load been retained uniformly by sediments throughout the lake.

Whatever the cause of sediment AEP increase in the northern control basin, statistical analysis of the contemporary AEP data revealed that there was no longer a significant difference in mean concentration between the two basins. Analysis of the contemporary sediment TP data did, however, confirm significantly higher concentrations in the central impact basin than in the northern control basin. It is possible that the increased concentration was a legacy of phosphorus-rich waste input from the fish cages between 1983 and 2009. Sediment TP includes the fractions represented by AEP as well as more inert forms of phosphorus. The discrepancy between sediment TP and AEP long-term

trends may be due to the mobility of AEP fractions. Labile, Fe-oxyhydroxide bound and organic phosphorus are readily released from the sediment to the water column (Boström *et al.* 1982; Søndergaard *et al.* 2003). High flushing rate coincident with a period of internal phosphorus loading will remove a portion of the released phosphorus from the lake, thus preventing it from being recycled back into the sediment and maintaining the AEP reservoir (Marsden 1989). On the other hand, less reactive apatite and metal-oxide bound phosphorus, also derived from the fish farm, are more likely to persist in the sediment and continue to enhance the concentration of total phosphorus in sediments from the central impact basin relative to the northern control.

The contemporary mean sediment TP concentrations of the northern control basin (2851 μg g^{-1} DW) and the central impact basin (3034 μg g^{-1} DW) are comparable to the range of concentrations measured in a survey of five English lakes, 1000 – 4010 μg g^{-1} DW (Davies 1996); and Loch Leven, Scotland, 2288 μg g^{-1} DW (Spears *et al.* 2006). The central impact basin of Esthwaite Water has the distinction of being the only one of these lakes to have contained aquaculture cages. Possibly as a consequence, it has the second highest mean sediment TP concentration after Windermere. Although none of the other lakes have been subjected to aquacultural operations, many of them have experienced prolonged periods of high external phosphorus loading from other sources and suffered poor water and ecological quality as a result, e.g. Windermere (McGowan *et al.* 2012), Bassenthwaite (Bennion *et al.* 2000; Thackeray *et al.* 2006) and Loch Leven (Spears *et al.* 2012). In the case of Loch Leven, external phosphorus inputs were reduced by 60 % between 1985 and 1995 to reverse water quality degradation (Bailey-Watts & Kirika 1999). The lake responded slowly and significant improvements in water quality were not observed until approximately 20 years later (D'Arcy *et al.* 2006). The prolonged recovery was attributed to the process of internal loading (Spears *et al.* 2012). Already, evidence suggests that the release of phosphorus accumulated in the sediment of Esthwaite Water has the potential to delay its recovery similarly (refer to Chapter Three for details).

The year following fish cage removal, Maberly *et al*. (2010) reported the annual mean TP concentration of the surface 5 m of the water column as 20.4 μg l^{-1}, which classified Esthwaite Water as having Moderate Ecological Status according to the EU WFD. Four years later, the epilimnion (0 – 5 m) annual mean TP concentrations of 22.3 μg l^{-1} (northern control basin) and 20.5 μg l^{-1} (central impact basin) would suggest that water quality had not improved since then. While the long-term monitoring data for Esthwaite Water does appear to show slight improvement in recent years, it was not determined to

be significant (refer to Chapter Three). Sas (1989) predicted that the implementation of restorative measures would induce net release for a period exceeding five years in lakes characterised by sediment TP concentration greater than 2500 μg g^{-1} DW. The desired water quality improvements may not become apparent in Esthwaite Water for many years if this prediction is realised.

Sediment TP concentration and AEP concentration increased significantly with increasing water depth. In both the northern control and central impact basin, the highest sediment phosphorus concentrations were measured at the deepest sampling sites. This pattern of sediment phosphorus distribution has been observed in a previous study of Esthwaite Water and was linked to sediment focussing processes (Mackay *et al.* 2012). Focussing describes the tendency of fine-grained sediment, to which phosphorus is closely associated (Nõges & Kisand 1999), to be concentrated in deeper parts of a lake. It occurs because the dominant sediment resuspension and transportation processes (e.g. gravity-driven slope failure, wave mixing and wind-driven currents) tend to be strongest near the water surface and decrease in strength with increasing depth. Sediment particles will be transported out of shallow areas until they reach a depth at which these processes are no longer sufficient to keep them suspended (Blais & Kalff 1995) and thus the more easily transported fine particles will be selectively moved to deeper areas of a lake.

4.4.2 Sediment phosphorus fractionation

The concentrations of each of the extracted phosphorus fractions did not differ substantially between contemporary sediment samples retrieved from the central impact basin and samples retrieved from the northern control basin. In the deep phosphorus-rich sediments, although the apportionment of the total phosphorus concentration between fractions was similar in the two basins, the concentration of each fraction was markedly higher in the central impact basin than in the northern control basin. However, the only significant difference was determined for the Ca-bound phosphate, extracted from the sediment by acid dissolution. This fraction comprises carbonate adsorbed phosphorus and apatite. The findings are consistent with the results of similar investigations, both in marine and freshwater environments. Previous investigations have used more elaborate sequential extraction techniques than the one used here to distinguish between different forms of apatite (e.g. detrital, authigenic or biogenic apatite). Biogenic apatite, which includes apatite originating from hard fish tissues (bones and teeth), has been measured at significantly higher concentrations in the sediment below active fish cages than in sediment from an unaffected control site (Jia *et al.* 2015; Matijević *et al.* 2008, 2012). It is

possible that hard fish tissues lost from the fish cages have been preserved in the sediment and continue to boost the apatite-bound phosphorus concentration of the central impact basin relative to the northern control basin. Phosphorus fractionation in contemporary sediment does not necessarily reflect fractionation during the period that cages were installed in the lake. In the years since fish farm closure variation in sediment biogeochemical characteristics and the uptake-release cycle of mobile phosphorus may have resulted in considerable redistribution of sediment phosphorus between the different solid phases.

Three different sediment phosphorus extraction techniques were used in this study; the sequential extraction of six different phosphorus forms (Psenner *et al.* 1988), the single-step extraction of AEP (Drake & Heaney 1987), and total phosphorus extraction by hot hydrogen peroxide – sulphuric acid digestion (Rowland & Grimshaw 1985). In theory, the sum concentration of the mobile phosphorus fractions (labile, reductant-soluble and organic phosphorus) determined by the sequential extraction should be comparable to AEP concentration. Likewise, the sum of all sequentially extracted fractions should be equal to the sediment TP concentration. However, there were significant differences between the outcomes of these different extraction methods when applied to sub-samples of the same sediment samples. The mean sediment TP concentration of sub-samples subjected to the sequential extraction procedure was significantly higher than the mean concentration of sub-samples that underwent hydrogen peroxide – sulphuric acid digestion. It appears that hydrogen peroxide – sulphuric acid digestion was the least effective of the two extraction methods, perhaps the reagent was not sufficiently strong or exposure time was too short.

The mean AEP concentration was higher than the mean concentration of sequentially extracted mobile phosphorus fractions. The molarity of NaOH solution used to dissolve the reductant-soluble phosphorus fraction during sequential extraction was higher than that of the NaOH solution used in the single-step AEP extraction. It is thought that a large pH increase during extraction with NaOH favours back precipitation of phosphorus liberated from Fe- or Mn-oxyhydroxide complexes onto $CaCO_3$ in the sediment (Golterman & Booman 1988). The re-adsorption of phosphorus during the sequential extraction procedure may explain the discrepancy in results between the two extraction procedures. The complexity of the sequential extraction procedure could also have had an impact. Whereas the results of the AEP extraction were fairly repeatable, the analytical error within each step of the sequential extraction scheme was relatively high. Alternatively,

storage-induced changes in phosphorus fractionation could be responsible for the differences. An investigation by Lukkari *et al*. (2007) found that phosphorus fractionation in sediment samples changed during storage and that the change became more pronounced as the storage time between sampling and analysis increased. The AEP extraction and sequential extraction were not conducted at the same time, the sequential extraction was conducted first by several months. Redistribution of sediment phosphorus in the period between extractions may be partially responsible for the difference between AEP concentration and the sum concentration of sequentially extracted mobile phosphorus fractions. To obtain more reliable results, it is recommended that sediment samples are stored in an N_2 atmosphere between sampling and analysis to prevent changes in phosphorus forms (Lukkari *et al.* 2007).

4.4.3 Potential phosphorus release mechanisms

Statistical analysis of contemporary sediment phosphorus data collected throughout 2014 did not detect significant temporal variations in the monthly mean concentrations of either AEP or sediment TP. Alone, these results imply that the monthly net flux of phosphorus between the sediment and water column was negligible over the sampling period. However, temporal variation in the water column TP concentration suggests differently. The TP concentration increased considerably in the hypolimnion of both basins over the summer and early autumn, and to a lesser extent in the metalimnion of the central impact basin only. A similar pattern was not observed in the monthly mean TP concentration of the epilimnion, which remained relatively stable throughout the year. If high external phosphorus loading was responsible for the TP increase it would also have been reflected in an increase in the epilimnion TP concentration over the same period. By deduction, the metalimnion and hypolimnion TP spikes between June and October must be attributable to the release of phosphorus from lake sediments. The amount of phosphorus required to raise hypolimnion TP to peak concentration was probably only a tiny fraction of the total mass of release-sensitive phosphorus stored in surface sediments. This is a common occurrence in shallow lakes and can be explained by considering the spatial variation in surface sediment characteristics in relation to the available phosphorus stock (Boström *et al.* 1988). A combination of analytical error at the phosphorus extraction stage and high spatial variation may have obscured the statistical detection of relatively small fluxes of sediment phosphorus that were sufficient to cause a noticeable increase in water column TP concentration.

The seasonal pattern of sediment phosphorus flux observed in Esthwaite Water is typical of many lakes and has largely been related to phosphorus release mechanisms driven by temperature and biological activity (Jensen & Andersen 1992; Søndergaard *et al.* 1999, 2013; Spears *et al.* 2007b). These include the stimulation of organic matter mineralisation and release of inorganic phosphate as temperature increases (Boström *et al.* 1982), and the increased sedimentation of organic material related to seasonal variations in phytoplankton productivity. In addition, the rate of oxygen supply to the sediment surface is often insufficient during periods of high organic loading to satisfy the oxygen demand of mineralisation processes (Walker 1979). The requirement for electron acceptors by the respiration of anaerobic bacteria produces a strongly reducing environment (Jones 1985). Under such conditions, the reduction of redox-sensitive phosphorus complexes (e.g. phosphorus adsorbed to Fe- and Mn-oxyhydroxides) further enhances sediment phosphorus release (Mortimer 1941, 1942). Significant positive correlations between water column TP and either air temperature or water temperature, and significant negative correlations between TP and DO saturation in both basins indicate that sediment phosphorus mobility in Esthwaite Water is, at least in part, regulated by temperature and associated biological processes.

Given that the apportionment of phosphorus between mobile fractions did not differ significantly between the central impact basin and northern control basin, it is unsurprising that the same release mechanisms appear to be prevalent in both. The only major difference found was that epilimnion TP concentration in the central impact basin correlated significantly to the cube of wind speed. Unexpectedly, this relationship was negative. A positive relationship between the two variables would have implied sediment phosphorus release due to wind-induced sediment resuspension, which would have been in-keeping with observations in the shallow, photic zones of other lakes, for example, Loch Leven (Spears *et al.* 2012) and Lake Arreso (Søndergaard *et al.* 1992). The relationship could be explained by the effect of wind-induced mixing on thermocline depth and consequently on redox conditions at the sediment-water interface. For example, when wind-induced mixing is low the thermocline may rise above 5 m depth, leading to the development of anoxic conditions within the shallow depth zone and the release of phosphorus from Fe-oxyhydroxide complexes (Gächter & Müller 2003). Conversely, periods of high wind-induced mixing maintain oxic conditions at the surface of shallow depth zone sediments, thereby preventing the anoxic diffusive release of phosphorus to the water column (Spears & Jones 2010).

4.5 Conclusion

The spatial variability of sediment phosphorus concentration during the period of fish farm operations in Esthwaite Water indicates that the release of waste from salmonid culture cages did enhance the phosphorus concentration of lake sediments. Five years after the removal of cages, sediment phosphorus concentrations in the impacted basin remained high. Unfortunately, it was not clear if this enrichment was a legacy of phosphorus input from aquacultural operations, or if other external phosphorus sources emerged following fish farm closure to sustain high sediment concentrations. Sequential extraction of phosphorus from contemporary sediment samples revealed that aquaculture has not had a substantial long-term effect on the fractionation of sediment phosphorus. Most phosphorus in contemporary sediment appears to be in readily mobile forms. Although statistical analysis of the monthly sediment phosphorus data did not determine significant temporal variation, the seasonality of hypolimnion TP concentration in 2014 strongly suggests that phosphorus was released from the sediment to the water column. The main period of presumed release was during the summer and appeared to be driven by temperature-controlled bacterial decomposition of organic matter and associated changes in redox conditions at the sediment-water interface. Closure of the fish farm in 2009 and the consequent cessation of phosphorus-rich waste input from culture cages was expected to instigate improvements in the water quality of Esthwaite Water. However, the continuation of internal phosphorus loading threatens to delay the recovery by many years.

Chapter Five

Modelling the response of phytoplankton communities in Esthwaite Water (UK) to changes in nutrient load, grazing pressure and air temperature

(Photo credit: I.J. Winfield)

In Chapter Three phosphorus availability was identified as the main driver of phytoplankton biomass change. In this chapter, the lake model PROTECH is used to investigate its importance relative to top-down control by zooplankton grazing. As climate warming and sediment phosphorus release were identified previously as factors that could delay lake recovery, PROTECH is also used to assess the potential future impact of increasing air temperature and persistent internal loading on Esthwaite Water.

5.1 Introduction

Throughout the 20th century, much ecological research and debate was focussed on determining the hierarchy of forces structuring ecosystems (Elton 1927; Hairston *et al.* 1960; Lindeman 1942; Oksanen *et al.* 1981). With respect to limnology, the bottom-up effect of nutrient availability was traditionally considered to be the major structuring factor in lakes (Persson et al. 1988, and references therein). An opposing view, for which the strength of evidence has increased in recent decades, is that the productivity and biomass of a certain trophic level may be regulated by predation or grazing from the level above (Carpenter *et al.* 1985; Shapiro *et al.* 1975), especially in shallow systems (Benndorf *et al.* 2002; Jeppesen *et al.* 1997). It is now widely accepted that the two processes are not mutually exclusive, top-down and bottom-up mechanisms can operate simultaneously (Leibold *et al.* 1997; McQueen *et al.* 1986; Pace *et al.* 1999). The focus of investigations has shifted towards determining the degree of interaction between these opposing regulatory mechanisms and how their relative importance might vary with global- and regional-scale environmental change (Bunnell *et al.* 2014; Jeppesen *et al.* 2003; Kerimoglu *et al.* 2013; Perga *et al.* 2010; Shurin *et al.* 2012).

At the regional level, human activity in lakes and catchments can enhance or inhibit top-down and bottom-up processes. The impact is often unintentional and occurs as a consequence of actions taken for another purpose. Unfortunately, the uncontrolled perturbation of either top-down or bottom-up forces has the potential to severely alter lake food web structure, and hence compromise the provision of essential ecosystem services (Folke *et al.* 2004; Holmlund & Hammer 1999; Smith 1998). Nutrient enrichment resulting from land use changes in the catchment is especially problematic as it prompts a series of undesirable bottom-up interactions (Harper 1992). Increased nutrient availability typically promotes the growth of phytoplankton, often including toxin-producing cyanobacteria, which can impose a cost in the supply of clean water for human consumption and restrict recreational activities at the lake (Chorus & Bartram 1999; Codd *et al.* 2005). The resultant algal blooms reduce light penetration, potentially limiting the growth of submerged macrophytes (Jones *et al.* 1983; Jupp & Spence 1977). Sedimentation of the readily degradable organic matter produced by the bloom stimulates microbial mineralisation at the sediment surface, thereby increasing the probability of hypolimnetic anoxia and the release of sediment-bound phosphorus to the overlying water column (Phillips *et al.* 1994; Ripl 1986). Populations of species less tolerant of reduced oxygen levels (e.g. salmonids) decline and, in extreme cases, the combination of

hypolimnetic hypoxia or anoxia and toxic algae has been reported to cause fish kills (Muller & Stadelmann 2004).

Since the implementation of the EU Water Framework Directive (WFD) in 2000, increasing efforts have been made to improve the ecological condition of eutrophic lakes (European Union 2000). However, it has become apparent that simply reducing the external causes of eutrophication does not guarantee lake recovery within the timeline stipulated by the EU WFD (Hering *et al.* 2010). In many lakes the effect of external nutrient load reduction is buffered by phosphorus release from the lake sediment, which serves to maintain high water column phosphorus concentrations, and consequently the potential for high algal biomass (Jeppesen *et al.* 2005; Phillips *et al.* 1994; Søndergaard *et al.* 2007; Spears *et al.* 2012). Phosphorus accumulates in the sediment during periods of high external loading. Removal or reduction of the external phosphorus load triggers a switch from net annual uptake to net annual release of phosphorus from sediments as the water column and surface sediment concentrations re-equilibrate (Sas 1989; Søndergaard *et al.* 1999). The longevity and importance of internal phosphorus load for overall lake phosphorus concentrations relate mainly to hydraulic retention time, sedimentation rate, loading history and the chemical characteristics of the sediment (Marsden 1989).

Biomanipulation has often been employed to combat the effects of eutrophication, either alongside or instead of the more traditional bottom-up approach of reducing external nutrient load (Jeppesen *et al.* 2012; Potthoff *et al.* 2008; Skov & Nilsson 2007; Søndergaard *et al.* 1990, 1997). This top-down remediation technique is based on the fundamental assumption that a change in predator biomass at the highest trophic levels of an aquatic food web cascades down to the lowest level (Shapiro *et al.* 1982). The abundance of planktivorous fish in a lake is reduced either by selective removal or by increasing the stock of piscivorous fish (Jeppesen *et al.* 2012). In turn, the predation pressure on zooplankton is relieved and grazing by this trophic level on phytoplankton increases. As fish are size-selective predators the zooplankton population will not only increase in response to a reduction in planktivorous fish, but the community structure may also shift towards larger-bodied individuals (e.g. *Daphnia*), presumably with higher grazing rates than the smaller species (e.g. *Bosmina*) that dominate when predation pressure is high (Brooks & Dodson 1965; Stenson *et al.* 1978). Under such conditions phytoplankton biomass should, in theory, decline. Indeed, marked improvements in water transparency and phytoplankton biomass have been recorded in many lakes immediately after food web manipulation (Potthoff *et al.* 2008; Søndergaard *et al.* 2008). However,

biomanipulation projects are frequently observed as having poor long-term effects, especially if nutrient loads to the lake remain high (Benndorf 1987; Gulati & van Donk 2002; Sayer *et al.* 2016).

Globally, climate change has the potential to alter the balance between the top-down and bottom-up mechanisms that determine lake ecosystem structure and function (Jeppesen *et al.* 2010; Shurin *et al.* 2012). It is well known that climate-induced alterations to the physical environment influence seasonal variability in lake community structure, and consequently the strength of top-down and bottom-up forces (Reynolds 1989; Sommer *et al.* 1986). It therefore seems likely that long-term climate change could have a considerable impact on the relative importance of top-down and bottom-up processes at the inter-annual scale as well. Already there is evidence that the differential effect of air temperature increase on the phenology and size spectra of interacting species disrupts predator-prey dynamics (Adrian *et al.* 2006; Dossena *et al.* 2012; Winder & Schindler 2004b; Yvon-Durocher *et al.* 2011).

Perhaps of greatest concern to lake managers is that rising temperatures tend to favour cyanobacteria over other phytoplankton (Kosten *et al.* 2012; Paerl & Huisman 2008). Cyanobacteria have certain ecophysiological traits that give them a competitive advantage in the nutrient-depleted, poorly-mixed water column conditions that typically prevail during the summer and early autumn (Carey *et al.* 2012). Many species of cyanobacteria produce gas vesicles (Walsby 1994), which allows buoyancy to be regulated so that cells can exploit light resources at the surface and nutrient resources at depth (Ganf & Oliver 1982; Reynolds *et al.* 1987). In addition, some genera (e.g. *Dolichospermum* and *Aphanizomenon*) are nitrogen fixers giving them a significant advantage over other taxa when the supply of dissolved nitrogen is limiting (Schindler 1977). Increased air temperature causes lakes to stratify earlier in spring and de-stratify later in autumn, thereby prolonging the optimal growth period for cyanobacteria (Wagner & Adrian 2009, 2011; Winder & Schindler 2004a). It is a widely held view that cyanobacteria are less susceptible to grazing by herbivorous zooplankton than other forms of algae (Ger *et al.* 2014; Visser *et al.* 2005). Consequently, the increased dominance of cyanobacteria caused by climate warming could diminish top-down control on total phytoplankton biomass. The promotion of potentially toxic cyanobacterial blooms caused by warming threatens to offset the positive impact of eutrophic lake remediation by nutrient load reduction (Wagner & Adrian 2009; Wilhelm & Adrian 2008) or biomanipulation (Jeppesen *et al.*

2010). It is important that we gain a thorough understanding of climate change impacts on food web dynamics in order to implement effective lake management strategies.

In this study, the process-based model PROTECH (**P**hytoplankton **R**esp**O**nses **T**o **E**nvironmental **C**hange) was used to examine how local environmental changes and global climate warming interact to affect phytoplankton population dynamics. Process-based models are grounded on a theoretical understanding of relevant ecological processes and provide a useful framework to investigate ecosystem response to variation of specific environmental conditions (Cuddington *et al.* 2013; Elliott *et al.* 1999). As a result, they offer a significant advantage in predicting the effects of environmental change compared to the statistical analysis of previously collected data, where it may be difficult to distinguish causal relationships among of a wide range of co-related ecological variables.

Esthwaite Water, a eutrophic lake in the English Lake District, was selected as the site to be modelled. It has long been prone to high algal biomass and summer cyanobacterial blooms, but efforts have been made in recent years to improve its ecological condition by reducing the external nutrient load (Maberly *et al.* 2011). The lake is currently stocked with triploid brown trout (*Salmo trutta*). Therefore, there is scope to enhance the effects of external nutrient load reduction by manipulating stocks to induce a trophic cascade on the phytoplankton community.

This investigation comprised two distinct, yet complementary, objectives:

(i) To determine, by way of a factorial experiment, the relative importance of top-down and bottom-up forces on seasonal- and annual-scale phytoplankton dynamics under a range of air temperature scenarios; and

(ii) To assess potential recovery trajectories and timescales in a eutrophic lake following external nutrient load reduction. Multi-year simulations of phytoplankton communities were conducted under a range of plausible scenarios, based upon different combinations of long-term air temperature increase and internal phosphorus load decline.

It was hypothesised that:

(i) Under current climatic conditions the relative importance of top-down and bottom-up processes would vary throughout the seasons with successional changes in phytoplankton community composition and zooplankton abundance.

(ii) Higher air temperature simulations would favour cyanobacterial growth on account of their ecophysiological adaptations, amplifying the bottom-up effect of nutrient load variability on overall phytoplankton biomass.

(iii) Long-term air temperature increases would negate the potential for internal phosphorus load decay to reduce total phytoplankton biomass by enhancing cyanobacterial growth.

5.2 Methods

5.2.1 Study site description

Esthwaite Water is a small lake (surface area 0.96 km^2, mean depth 6.4 m, maximum depth 16 m) located in Cumbria, UK. It has undergone persistent eutrophication since the late 1960s (Dong *et al.* 2011; Talling & Heaney 1983) and regularly produces algal blooms in the summer and early autumn consisting mainly of *Dolichospermum* and *Aphanizomenon* (Heaney *et al.* 1992). In recent years, efforts have been made to improve the ecological and water quality of Esthwaite Water to meet the criteria established by the EU WFD. Historical sources of nutrient enrichment include treated sewage effluent from the local Wastewater Treatment Works (WwTW), fertiliser run-off from surrounding agricultural land and waste from salmonid culture cages installed in the lake. The nutrient load from aquaculture was completely removed in 2009 with the cessation of fish farm activities, although the lake does continue to be stocked with *c*. 7,500 brown trout annually for game fishing purposes. Additionally, in 2013 major upgrades to waste handling processes at Hawkshead WwTW were completed, reducing the load of phosphorus from this source by *c*. 60 % (refer to Chapter Three for details). Despite the significant reduction in external nutrient load, it is speculated that high phytoplankton productivity will be maintained for some time due to the release of phosphorus from lake sediments into the overlying water column (Drake & Heaney 1987; Mackay *et al.* 2012).

5.2.2 PROTECH model description

PROTECH is a process-based model that simulates the daily growth of multiple phytoplankton genera throughout the water column. PROTECH has been developed and tested on a wide range of lakes and reservoirs around the world over the last two decades and been used in multiple peer-reviewed publications (for an overview, refer to Elliott *et al*. 2010). The core biological component of PROTECH is an equation that determines the daily change in the chlorophyll *a* concentration ($\Delta X/\Delta T$, $\mu g\ l^{-1}\ d^{-1}$) of each taxa of phytoplankton included in the model:

$$\Delta X/\Delta T = (r' - S - G - D).X \quad (5.1)$$

where, r' is the growth rate (d^{-1}) defined as a proportional change over 24 h, S is the loss rate due to settling out of the water column, G is the loss rate caused by *Daphnia* grazing, D is the loss rate due to dilution and X is the initial starting concentration of chlorophyll *a* ($\mu g\ l^{-1}$). The growth rate is calculated from:

$$r' = \min\{r'_{(\theta,I)}, r'_P, r'_N, r'_{Si}\} \quad (5.2)$$

where, $r'_{(\theta,I)}$ is the growth rate determined by temperature and daily photoperiod, and r'_P, r'_N, r'_{Si} are the growth rates determined by phosphorus, nitrogen and silicon if their concentrations are less than 3, 80 and 500 $\mu g\ l^{-1}$, respectively (Reynolds 1984a). The r' values are taxa dependent (e.g. non-diatoms are not limited by silica concentrations below 500 $\mu g\ l^{-1}$) and relate to the morphology of the phytoplankton. For each taxon within the model, the initial starting value of chlorophyll *a* (X $\mu g\ l^{-1}$) is adjusted on a daily time step to predict the change in chlorophyll *a* concentration for each layer in the water column.

Phytoplankton population dynamics are subject to mortality as a consequence of sinking out to deeper, darker water and of consumption by pelagic grazers. PROTECH is constructed to estimate the scale of these losses on extant populations. Biomass loss as a result of grazing by zooplankton is determined by the temperature and food-dependent growth and reproduction relationships for *Daphnia* developed by Reynolds (1984b). The temporal grazing dynamics of the *Daphnia* can be expressed thus:

$$rG = \Delta G/\Delta T \quad (5.3)$$

where, G is the grazing pressure expressed as the amount of edible chlorophyll *a* (X_{ed}) consumed each time iteration ($\mu g\ l^{-1}\ d^{-1}$) and r is the daily rate of change of G and is dependent upon X_{ed} and water temperature. It is possible to apply this grazing routine only to certain alga included in the model. In this case, all of the cyanobacteria genera were exempt from grazing by *Daphnia*. Cyanobacteria are generally considered to be low-quality food for zooplankton grazers for several reasons: (i) filamentous or colonial morphologies can clog filtration appendages (DeMott *et al.* 2001; Gliwicz & Lampert 1990), (ii) inadequate nutritional composition inhibits the growth and reproduction of consumers (Ahlgren *et al.* 1990; Gulati & DeMott 1997; Müller-Navarra *et al.* 2000), and (iii) toxic metabolites can be produced (Fulton & Paerl 1987; Lampert 1982). The model assumes that consumed phytoplankton return all of their component nutrients back to the water instantaneously.

The model also includes a genera-specific movement function that calculates the depth of each alga in the water column, accounting for the movement of the water and Stokes' Law (sinking down the water column), as well as the motility and buoyancy of some phytoplankton (e.g. positive movement up the water column, dependent upon light intensity for motile genera). Nutrient concentrations in the water column are modified to reflect uptake determined by growth and daily supply and loss via inflow-outflow exchange. For all simulated phytoplankton, it is assumed that these nutrients are consumed from the water column following a stoichiometric ratio of 82 g silica (only for diatoms): 8.3 g nitrogen: 1.2 g phosphorus: 1.0 g chlorophyll (Stumm & Morgan 1996).

The physical side of the model divides the simulated waterbody into 0.1 m depth strata, which are adjusted in volume and surface area to reflect the morphology of the basin. An initial profile of the water column comprising starting temperature, nutrient concentrations, and an inoculum of each phytoplankton genus is defined for day one (equivalent to 1st January in the simulations presented here). Daily wind speed, cloud cover, inflow discharge and nutrient concentration, and outflow discharge data are input to the model and daily insolation is varied depending upon the day of the year and latitude.

For each 24 h time step, the vertical extent of the mixed layer is calculated as a function of heat fluxes and wind-induced stirring following the Monin-Obukhov equation, which assumes initial uniformity. Resistance of the existing density structure to mixing is determined by the Wedderburn Number, and the intensity of stratification is modified accordingly (Imberger & Hamblin 1982). Below the thermocline, extra mixing is provided by eddy diffusivity (K_d), which is set at a constant value derived from the lake's maximum depth (Elliott & Thackeray 2004). Water column profiles of temperature, nutrient concentrations and phytoplankton composition are changed at the start of each time step as a result of mixed layer depth and diffusivity variation. The biological functions described above (Equations 5.1, 5.2 and 5.3) are then used to calculate the new chlorophyll *a* and dissolved nutrient concentrations of the top 5 m of the water column at the end of the time step (24 h), assuming no further vertical movement. For full details of the theoretical basis and formulations of PROTECH, refer to Reynolds *et al*. (2001).

5.2.3 Driving data

The driving and validation data for the simulations were all taken from the long-term monitoring programme at Esthwaite Water (1945 to present) conducted by the Freshwater Biological Association (pre-1989) and Centre for Ecology & Hydrology (post

1989). Observed data from 2014 was specifically used as a basis for the simulations. This was deemed to be a typical year with respect to the seasonality of phytoplankton growth, and during this year detailed nutrient and discharge data were available for most of the major inflows to the lake.

Daily mean discharge data for the outflow (Cunsey Beck) were available for every day of the modelled period (Environment Agency 2015). Unlike the outflow, the discharge of the main inflow (Black Beck) and five smaller tributaries are not measured routinely. For the purpose of this study, discharge was measured at these sites on a monthly basis throughout 2014. Flow velocity (m s^{-1}) measurements were taken using a current meter (C2 Current Meter, OTT Hydromet GmbH, Kempten, Germany) and multiplied by stream cross-sectional area (m^2) to obtain discharge (m^3 s^{-1}). A strong correlative relationship was established between Black Beck discharge and Cunsey Beck discharge (r = 0.75, p < 0.001). This correlative relationship was used to obtain daily values of discharge for Black Beck to be included in the model. On average, Black Beck accounted for 57 % of the hydrological input to the lake. The sewage effluent discharge from Hawkshead WwTW and the volume of rain that fell directly over the lake were also taken into account. The hydrological budget was then balanced by ascribing the remaining unaccounted-for proportion of outflow discharge to the five minor inflowing tributaries.

Soluble reactive phosphorus (SRP) and nitrate concentrations were measured at 14 day intervals for the WwTW effluent and main inflow, and monthly for the five smaller inflows. Samples were filtered immediately upon collection, SRP was analysed according to Murphy & Riley (1962, refer to Section 4.2.4 for details) and nitrate concentration was determined by an ultraviolet spectrophotometric technique adapted from Armstrong (1963). In addition to SRP and nitrate, fortnightly silica measurements were also available for the main inflow [NERC Centre for Ecology & Hydrology (CEH) 2015]. Given the homogeneity of catchment geology (Fryer 1991), the silica concentration of the five smaller inflowing tributaries was assumed to be the same as for Black Beck. Daily nutrient concentrations for the inflows were obtained by linearly interpolating between the measured values. Although rainfall SRP concentration was not determined during the course of this investigation it was considered that rainfall directly over the lake would have been a significant source of phosphorus to Esthwaite Water. Based on the findings of previous studies at nearby sites rainfall SRP concentration was set at 25 µg l^{-1} (May *et al.* 1997). The input of phosphorus via rainfall over the wider catchment area was accounted for in the inflow nutrient concentrations.

PROTECH does not include equations to simulate the release or uptake of phosphorus from sediment, known to be an important component of the phosphorus cycle in Esthwaite Water (refer to Chapter Three). Sediment phosphorus flux (kg) was calculated from observed data using the following mass balance equation:

$$\text{Sediment Phosphorus Flux} = \left(M_{\text{TP\_2}} - M_{\text{TP\_1}}\right) - \left(TP_{\text{in}} - TP_{\text{out}}\right) \qquad (5.4)$$

where, $M_{\text{TP\_1}}$ is the phosphorus mass (kg) in the lake at the start of the period of interest and $M_{\text{TP\_2}}$ is the mass at the end of the period. TP_{in} is the mass (kg) of phosphorus added to the lake via external sources (e.g. inflows, rainfall, WwTW) and TP_{out} is the mass (kg) that is lost via the outflow. Throughout 2014 depth profiles (2 m intervals throughout the water column) of total phosphorus concentration were measured approximately monthly at the deepest point in the lake. Based on annual mid-summer profiles of water column temperature, a thermocline depth of 5 m was used. A volume-weighted average of total phosphorus concentration was calculated for the epilimnion (< 5 m depth) and hypolimnion (> 5 m depth). These concentrations were then multiplied by the corresponding volume of water to obtain the phosphorus mass contained within each depth zone on the day of sampling. Epilimnion and hypolimnion sediment phosphorus flux were calculated separately for the period between each sampling date and converted to a discrete daily flux (μg l^{-1} d^{-1}) for inclusion in the model. The reason for this separation was because the water column is typically thermally stratified between May and September each year (George & Hewitt 1999). It was assumed that during the period of stratification kinetic energy input from inflowing water will not be sufficient to cause mixing of the warm, less dense surface layer with the cooler, denser water below the thermocline (Nürnberg 1984). The inflow-outflow exchange of water and phosphorus is therefore restricted to the epilimnion. To account for this, the (TP_{in} – TP_{out}) component of the equation was omitted from calculations of hypolimnion internal load during the summer. The sediment phosphorus flux input to the model for each approximately month-long period between sampling dates is illustrated in Figure 5.1.

Daily meteorological data were drawn from two sources: wind speed and air temperature were measured by a buoy-mounted meteorological station on Esthwaite Water, while cloud cover (octas) estimates and rainfall (mm d^{-1}, used for the calculation of rainfall phosphorus load) were obtained from a citizen scientist who makes meteorological observations about 8 km north of the lake at Ambleside.

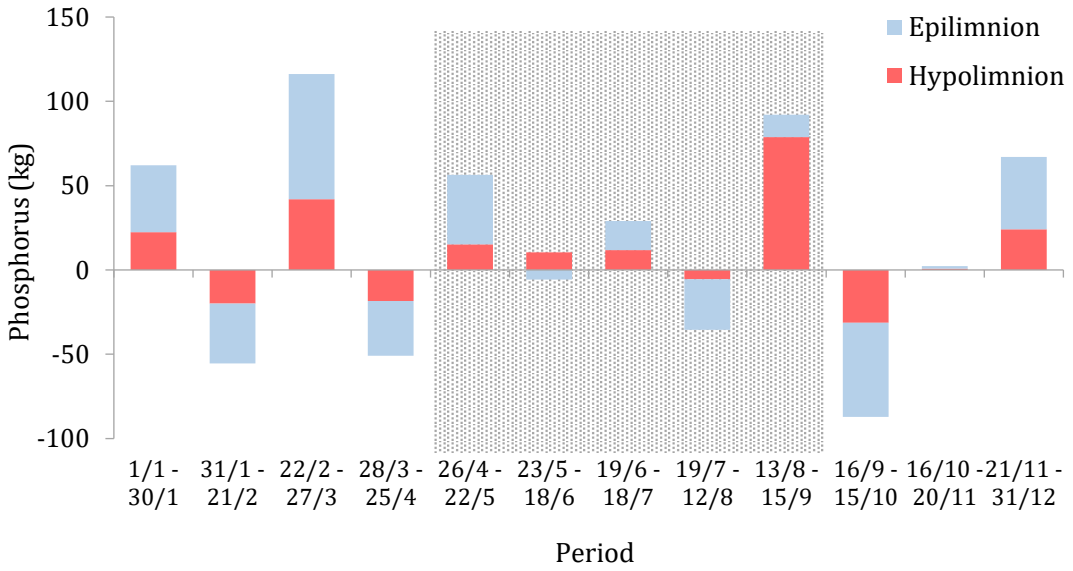

Figure 5.1: Mass of phosphorus exchanged between the water column and lake sediment for 12 approximately month-long periods throughout 2014. The total mass is the sum of the phosphorus masses exchanged across the sediment-water interface at < 5m depth (epilimnion) and > 5 m depth (hypolimnion). Positive values represent a release of phosphorus from the sediment to the overlying water column. Negative values indicate an uptake of phosphorus by the sediment. The grey box shows the typical timing of thermal stratification.

5.2.4 Validation of the baseline simulation

Fortnightly phytoplankton species counts and measurements of lake water temperature, SRP, nitrate, silica and chlorophyll *a* concentrations from an integrated sample of the upper 5 m of the water column were available for Esthwaite Water (NERC CEH, 2015). Examination of the phytoplankton count data revealed the numerically abundant phytoplankton taxa detected in the lake during 2014. This information was used to select the following PROTECH phytoplankton for the simulations: *Dolichospermum, Aphanizomenon, Aphanothece, Planktothrix*, *Asterionella, Chlorella, Plagioselmis* and *Chlamydomonas*. The phytoplankton were divided into taxonomic groups, with the exception of *Plagioselmis* and *Chlamydomonas* which were grouped together as flagellates (Table 5.1). The PROTECH instructions governing the vertical movement of the eight modelled phytoplankton are shown in Table 5.2.

Table 5.1: The morphological and phylogenetic characteristics of the eight simulated phytoplankton. The last two columns denote simple logic statements (True/False) which, if True, activate relevant functions in PROTECH.

Group	Phytoplankton	Surface Area (μm^2)	Cell Volume (μm^3)	Maximum Dimension (μm)	Grazed	Nitrogen Fixer
Cyanobacteria	*Dolichospermum*	6,200	29,000	75	F	T
	Aphanizomenon	5,200	15,400	125	F	T
	Aphanothece	7,854	65,450	500	F	F
	Planktothrix	7,350	13,970	300	F	F
Diatoms	*Asterionella*	6,690	5,160	130	T	F
Green Algae	*Chlorella*	50	33	4	T	F
Flagellates	*Chlamydomonas*	112	60	5	T	F
	Plagioselmis	108	72	11	T	F

Table 5.2: Summary of PROTECH instructions governing the vertical movement of the eight modelled phytoplankton.

Phytoplankton	Light Condition ($\mu mol\ photon\ m^{-2}\ s^{-1}$)	Movement ($m\ d^{-1}$)
Buoyancy-regulating cyanobacteria		
Dolichospermum and *Aphanizomenon*	≤ 10	Rise 0.1
	> 10 but ≤ 30	Nona
	> 30 but ≤ 100	Sink 0.1
	> 100	Sink 0.3
Aphanothece	≤ 10	Rise 0.1
	> 10 but ≤ 30	None
	> 30	Sink 0.1
Planktothrix	≤ 10	Rise 0.1
	> 10 but ≤ 30	None
	> 30	Sink 0.1
Non-buoyant, non-motile diatoms		
Asterionella	≤ 500	Sink 0.2
	> 500	Sink 1.0
Non-buoyant, non-motile green algae		
Chlorella	All light conditions	Sink 0.1
Swimming flagellates		
Chlamydomonas and *Plagioselmis*	≤ 30	Rise 0.5
	> 30 but ≤ 100	None
	> 100	Sink 0.5

PROTECH was run using the 2014 driving data described above. To validate the baseline simulation, visual comparison and Pearson product-moment correlation coefficients (*r*) were used to compare the simulated chlorophyll *a*, nutrient and water temperature data with the fortnightly measurements. It was necessary to convert the species count data from cells per ml to μg chlorophyll *a* l^{-1} in order to make this comparison. These values were calculated using the cell volume to chlorophyll *a* relationships given in Reynolds (1984b) and summed for the dominant taxa in the lake. Chlorophyll *a* concentration is reported throughout as a widely-used proxy of phytoplankton biomass.

5.2.5 Testing the combined effects of nutrient load, grazing rate and air temperature variation on the phytoplankton community

The baseline simulation described above was used to test the combined effect of top-down and bottom-up pressures on the phytoplankton community. This was achieved by repeatedly running the 2014 simulation through a step-wise combination of changes in nutrient load (both inflow nutrient concentrations and sediment-water phosphorus flux) and grazing rate (*G*). The multiplier for both factors was increased in 0.25 increments from zero to two resulting in a combination of 81 different simulations, including the initial validation run. As PROTECH does not incorporate *Daphnia* losses by predation into the grazing rate routine, the grazing rate multiplier used here acts as a manipulation of *Daphnia* population size by planktivorous fish. Set below one (baseline conditions), the grazing rate multiplier reduces grazing pressure on the phytoplankton, effectively producing a scenario of increased planktivorous fish biomass. At the other end of the range, high multipliers are consistent with a reduction in planktivorous fish biomass and consequently an increase in the grazing pressure exerted on phytoplankton by *Daphnia*.

For each permutation of the model, the annual, spring (March to May), summer (June to August), autumn (September to November) and winter (January to February, plus December) mean chlorophyll *a* concentrations were calculated by averaging daily chlorophyll *a* data for total biomass and for each phytoplankton taxonomic group separately. In addition, the annual and seasonal mean percentages of total phytoplankton abundance accounted for by cyanobacteria were calculated. The impact of nutrient load and grazing rate variations on phytoplankton biomass and community structure was determined by visual analysis of response variable surface plots.

To test the impact of changing air temperature on the mechanisms that control phytoplankton biomass and community structure, the 81 scenarios described above were

carried out a further three times with the air temperature manipulated to be 1, 2 or 3 °C higher than the original temperature. Natural patterns of air temperature seasonality were maintained in each simulation but centred around the higher annual mean. As before, the seasonal and annual means of total chlorophyll *a* concentration and cyanobacteria chlorophyll *a* were calculated for each of the modelled scenarios. Annual and seasonal mean values of non-cyanobacteria chlorophyll *a* concentration were calculated from the sum of diatom, flagellate and green algae chlorophyll *a* concentrations. General linear models were used to test for significant effects of air temperature variation and its interaction with grazing rate and nutrient load on annual and seasonal means of phytoplankton biomass. Significant effects were determined by a p value < 0.05. Statistical analyses were conducted using R version 3.2.3 for Windows (R Core Team 2016). The boxcox function from the MASS package for R was used to select the appropriate power transformation for non-normal data (Ripley *et al.* 2016).

5.2.6 Long-term projections of the phytoplankton community

PROTECH was run for a series of consecutive 30-year periods in order to predict potential long-term trends in phytoplankton biomass and community structure following the recent reduction of external nutrient load to Esthwaite Water. To investigate the importance of internal loading longevity on long-term phytoplankton trends, and consequently the lake recovery timeline, sediment phosphorus fluxes calculated for 2014 (Section 5.2.3) were extended over the 30-year modelling period under five different decay rate scenarios: 0, 5, 10, 25, and 50 % per annum (Figure 5.2a). To account for the seasonality of some important processes that influence phosphorus exchange between water and sediment [e.g. sediment redox conditions (Mortimer 1941, 1942)], and the retention of phosphorus in lakes [e.g. flushing rate (Marsden 1989)], hypolimnion fluxes for the periods between May and September were not subjected to annual reductions over the course of the simulation. Thus, internal phosphorus load to the hypolimnion during stratification was maintained at 111 kg each year of the simulation.

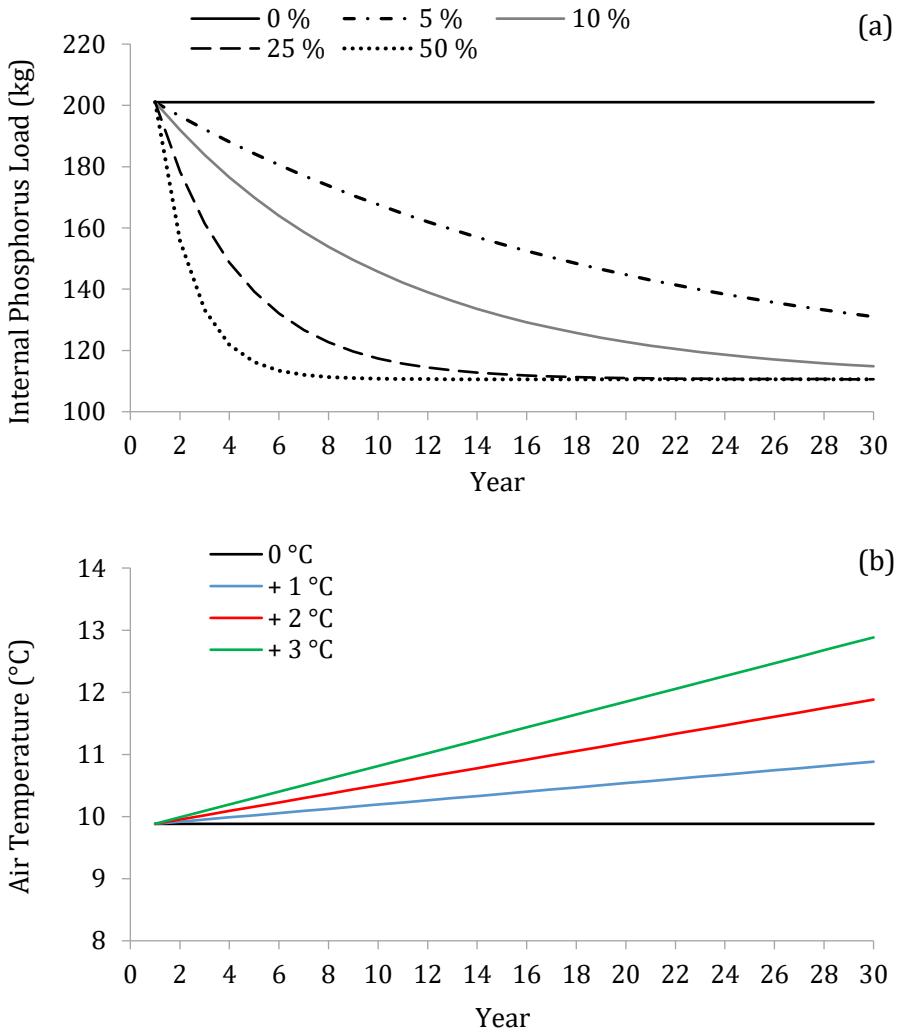

Figure 5.2: Trajectories of PROTECH input data used for the 30-year simulations of phytoplankton community. (a) Annual net internal phosphorus load. Legend denotes the percentage decrease in load each year. (b) Annual mean air temperature. Legend denotes the change in annual mean air temperature between the first year of the simulation and the final year of the simulation.

The impact of air temperature on phytoplankton was assessed by forcing the annual mean air temperature to increase by 1, 2 and 3 °C over the 30-year modelling period (Figure 5.2b). While the annual mean gradually increased over time, the seasonal air temperature pattern observed in 2014 was maintained for each year of the simulations. Based on the Intergovernmental Panel on Climate Change (IPCC) predictions of future climate warming these rates of air temperature increase range from realistic to extreme for the modelled period (IPCC 2014a). Each temperature change permutation was modelled five times; once for each version of sediment phosphorus flux decay. With the exception of sediment phosphorus flux and air temperature, the driving data were maintained at 2014 values year-on-year throughout all of the simulations.

For each of the long-term phytoplankton simulations, annual mean modelled chlorophyll *a* concentration was calculated from the geometric mean (the mean of the Log_{10} chlorophyll *a* concentrations converted back to unlogged concentrations), and corrected for the effect of the geometric calculation using the following formula:

$$\text{Modelled Chlorophyll } a = GM_{\text{chl}} * e^{0.5 \times (2.323 \times SD_{\text{g}})^2} \quad (5.5)$$

where, GM_{chl} is the geometric mean of simulated chlorophyll *a* concentrations, SD_{g} is the standard deviation from a population of UK lakes (WFD-UKTAG 2008). For lakes with moderate alkalinity (200 to 1000 μEq l^{-1}), such as Esthwaite Water, SD_{g} has a value of 0.345 (Maberly *et al.* 2016). WFD assessments use an annual mean of chlorophyll *a* calculated from the geometric mean (i.e. observed chlorophyll *a*) as it diminishes the effect of significantly outlying values, which might bias the average if the arithmetic mean were calculated instead. Therefore, calculation of annual mean modelled chlorophyll *a* in this manner allows for comparison against the EU WFD ecological status boundaries specific to Esthwaite Water and for the determination of possible timelines of lake recovery based on these legislative criteria. Annual mean cyanobacteria and non-cyanobacteria (i.e. diatoms, green algae and flagellates) chlorophyll *a* concentrations and percentage dominance by cyanobacteria were also calculated. In addition, the number of days per annum that cyanobacteria chlorophyll *a* concentration exceeded the World Health Organisation (WHO) moderate human health risk threshold of 10 μg l^{-1} was calculated (Chorus & Bartram 1999).

5.3 Results

5.3.1 Validation of the baseline simulation

For validation, PROTECH was run using driving data collected throughout 2014 and the output compared to measurements of phytoplankton chlorophyll *a*, nutrients and lake temperature from the same period. The simulation of surface water temperature was strongly correlated to the measured values (r = 0.99, p < 0.001). The only notable deviation from the observed data was a slight overestimation around day 290 (Figure 5.3a). Seasonal variation in simulated total chlorophyll *a* concentrations was consistent with the observed 2014 monitoring data (Figure 5.3b, r = 0.90, p < 0.001). Both cyanobacteria (r = 0.88, p < 0.001) and diatom (r = 0.88, p < 0.001) chlorophyll *a* simulations also proved to be a good fit to the observed data (Figures 5.3c/d).

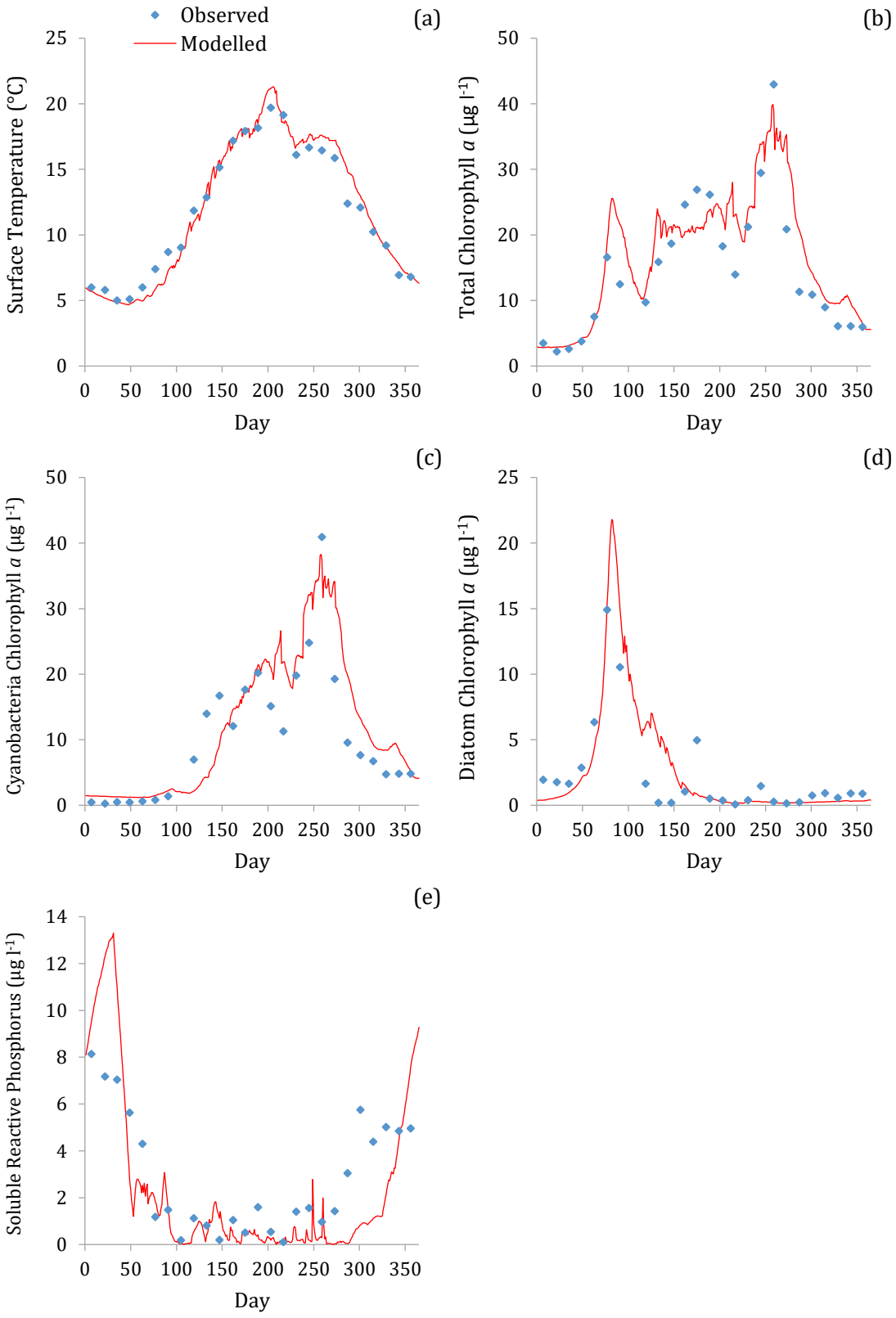

Figure 5.3: Comparison between observed (blue diamonds) and PROTECH simulated (solid red line) data for the surface 5 m of Esthwaite Water 2014: (a) water temperature, (b) total chlorophyll *a*, (c) cyanobacteria chlorophyll *a*, (d) diatom chlorophyll *a*, and (e) soluble reactive phosphorus.

Simulated lake nutrient data corresponded reasonably well to seasonal patterns observed in the field. Correlations between observed and simulated nitrate and silica concentrations were strong, $r = 0.82$ and 0.91, respectively, both $p < 0.001$. Simulated SRP concentrations were broadly in agreement with the measured values ($r = 0.80$, $p < 0.001$). However, the values simulated in January and December were markedly higher than observed and the model failed to capture the autumn SRP peak (Figure 5.3e). The discrepancies in simulated SRP concentration occurred out with the main phytoplankton growing season when meteorological factors, such as light attenuation and air temperature, are more likely to limit productivity. Overall, this simulation was considered adequate for use as a baseline for scenario testing.

5.3.2 Combined effects of nutrient load and grazing rate variation on the phytoplankton community

Predicted annual mean total chlorophyll *a* ranged from 3 – 23 μg l^{-1}, with the highest concentrations occurring under conditions of high nutrient load and low grazing rate (Figure 5.4a). The response of total chlorophyll *a* to nutrient load changes was greater than the response to variation in grazing pressure. The effect of grazing rate on total chlorophyll *a* was enhanced slightly at higher nutrient loads. Changes in grazing pressure and nutrient load had varying effects on the annual mean chlorophyll *a* levels of the taxa that were modelled.

Annual mean diatom chlorophyll *a* displayed a pattern of response similar to that of total chlorophyll *a* (Figure 5.4c). Diatom biomass responded positively to increasing nutrient load and decreasing grazing rate. However, grazing rate variation had more of an impact on this specific taxonomic group than it did on phytoplankton as a whole. The gradient of diatom chlorophyll *a* increase with decreasing grazing rate was greater than that of total chlorophyll *a*. Cyanobacteria were the dominant group, accounting for 40 – 74 % of annual phytoplankton biomass (Figure 5.4f). The response of cyanobacteria differed from the other algal groups in that the highest chlorophyll *a* concentration occurred when both nutrient load and grazing rate were high. Increases in cyanobacteria chlorophyll *a* were heightened as nutrient load and grazing rate increased (Figure 5.4b). Both green algae and flagellates responded more strongly to variation in grazing rate than they did to variation in nutrient load. The chlorophyll *a* concentration of both groups varied little in response to changing nutrient load when the grazing rate was above baseline conditions (Figure 5.4d/e).

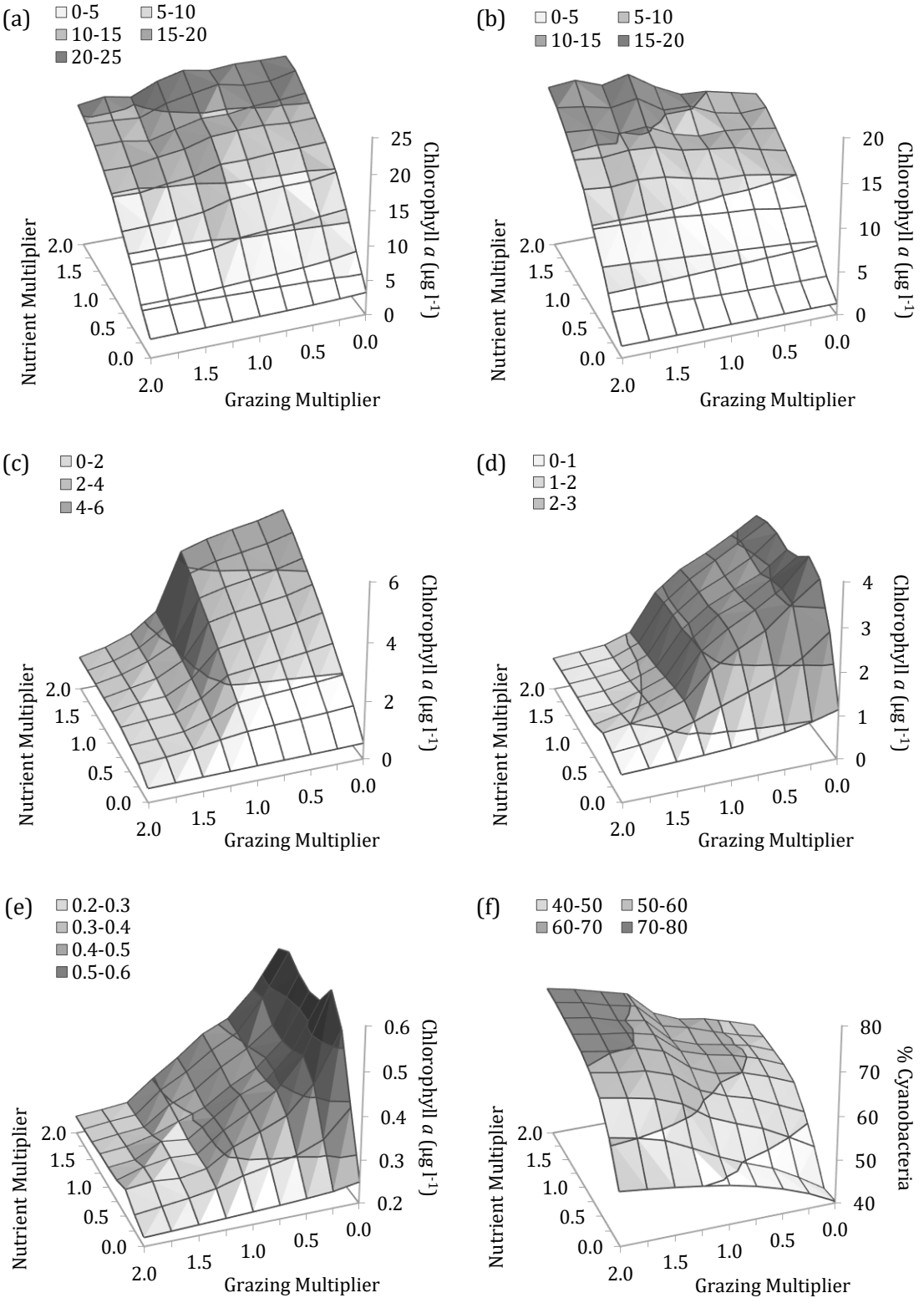

Figure 5.4: Annual mean chlorophyll *a* and percentage cyanobacteria abundance results, derived from 81 different scenarios of nutrient load and grazing rate: (a) total phytoplankton chlorophyll *a*, (b) cyanobacteria chlorophyll *a*, (c) diatom chlorophyll *a*, (d) flagellate chlorophyll *a*, (e) green algae chlorophyll *a*, and (f) percentage of total phytoplankton biomass accounted for by cyanobacteria.

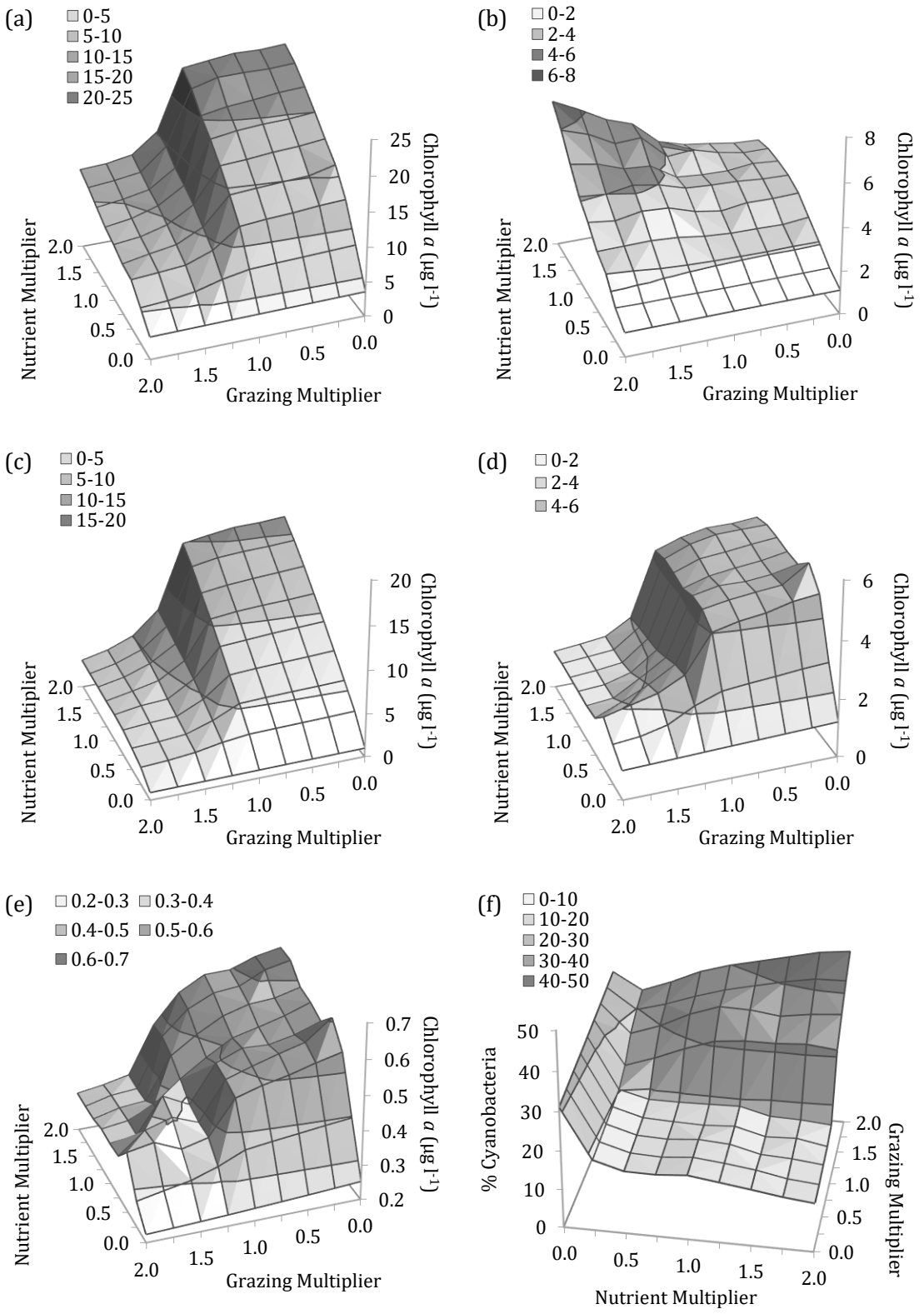

Figure 5.5: Spring (March to May) mean chlorophyll *a* and percentage cyanobacteria abundance results, derived from 81 different scenarios of nutrient load and grazing rate: (a) total phytoplankton chlorophyll *a*, (b) cyanobacteria chlorophyll *a*, (c) diatom chlorophyll *a*, (d) flagellate chlorophyll *a*, (e) green algae chlorophyll *a*, and (f) percentage of total phytoplankton biomass accounted for by cyanobacteria.

The spring (March to May) mean of total chlorophyll *a* increased with increasing nutrient load and decreasing grazing rate (Figure 5.5a). This pattern was reflected in the chlorophyll *a* of diatoms, the dominant algal group under baseline conditions (Figure 5.5c). As grazing rate and nutrient load increased there was a shift away from diatom dominance in the spring towards cyanobacteria dominance (Figure 5.5f). Even under such conditions, cyanobacteria levels remained relatively low (Figure 5.5b). Spring mean cyanobacteria concentration peaked at 7 μg l^{-1} compared to a diatom maximum of 16 μg l^{-1}. Flagellates accounted for a significant portion of spring algal biomass. Flagellate chlorophyll *a* levels increased rapidly with increasing nutrient load and decreasing grazing rate to plateau at 4 – 5 μg l^{-1} for scenarios where the grazing rate multiplier was below the baseline and the nutrient load multiplier above it (Figure 5.5d). The contribution of green algae to the total biomass was negligible. There was a tendency for the model to produce more green algae for scenarios that combined high nutrient load and low grazing rate (Figure 5.5e).

The predominant driver of summer (June to August) mean total chlorophyll *a* was nutrient availability. Although the total algal biomass showed a slight negative response to increased grazing pressure at low nutrient loads, the effect declined as the nutrient input increased. Total chlorophyll *a* ranged from 2 μg l^{-1} when nutrient load was at its lowest, to 39 μg l^{-1} when the baseline nutrient load was doubled (Figure 5.6a). The underlying cause for this pattern of response to nutrient load variation was cyanobacteria dominance in 79 of the 81 scenarios tested, on average they accounted for 76 % of the total summer mean biomass (Figure 5.6b/f). The response of cyanobacteria biomass to changes in grazing rate differed from that of total phytoplankton biomass in that cyanobacteria chlorophyll *a* levels increased with increasing grazing rate, and this relationship strengthened as nutrient load increased. Even so, the impact of nutrient load on cyanobacteria was far greater than that of zooplankton grazing.

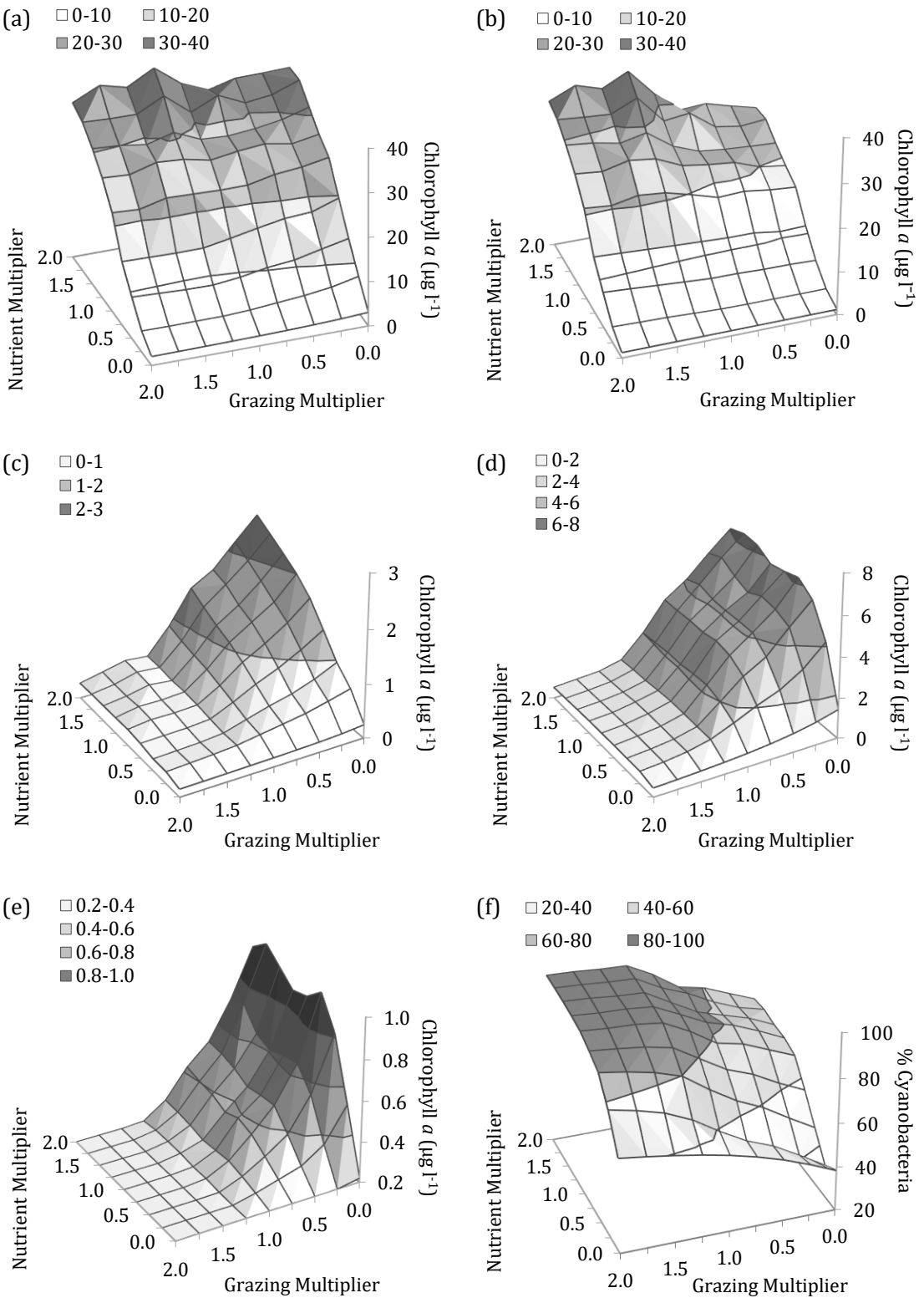

Figure 5.6: Summer (June to August) mean chlorophyll *a* and percentage cyanobacteria abundance results, derived from 81 different scenarios of nutrient load and grazing rate: (a) total phytoplankton chlorophyll *a*, (b) cyanobacteria chlorophyll *a*, (c) diatom chlorophyll *a*, (d) flagellate chlorophyll *a*, (e) green algae chlorophyll *a*, and (f) percentage of total phytoplankton biomass accounted for by cyanobacteria.

In contrast to cyanobacteria, summer flagellate biomass was strongly influenced by grazing rate and the impact of nutrient load variation was most pronounced when grazing was low. Summer flagellate chlorophyll *a* reached a maximum of 6 μg l^{-1} (Figure 5.6d). Summer flagellate levels did not vary greatly from those simulated during the spring. For all of the simulations, the diatom biomass was noticeably lower in the summer than it had been during spring. Summer diatom chlorophyll *a* remained relatively low (< 1 – 3 μg l^{-1}) regardless of driving conditions; biomass peaked in the scenario that combined the highest nutrient load and lowest grazing rate (Figure 5.6c). Again, green algae contributed little to the total biomass, a maximum summer mean of 1 μg l^{-1} was recorded when the grazing rate multiplier was set to zero and the baseline nutrient load was multiplied by 1.75 (Figure 5.6e).

Total chlorophyll *a* values remained high in the autumn (September to November) ranging from 3 – 30 μg l^{-1}. Again, cyanobacteria were the most abundant group accounting for 47 – 96 % of the total phytoplankton biomass (Figure 5.7f). The patterns of response in total chlorophyll *a* and cyanobacteria chlorophyll *a* were both very similar to those observed in the summer, with the exception that total chlorophyll *a* also increased in response to increasing grazing pressure at all nutrient load levels (Figures 5.7a/b). The biomass of the other three algal groups was considerably lower than in the summer and all showed less sensitivity to grazing rate and nutrient load in autumn than they had done during the previous season. Green algae and flagellates were most abundant under conditions of low grazing pressure and low nutrient load (Figures 5.7d/e). Diatoms peaked at 1 μg l^{-1} when the grazing rate was low and nutrient load high (Figure 5.7c). Green algae, flagellate and diatom levels dropped dramatically in the winter (January to February, December) and proved to be less responsive to variation in the drivers than at any other time of the year (Figures 5.8c-e). Total algal biomass (Figure 5.8a) was almost entirely comprised during the winter by cyanobacteria (Figures 5.8f). Winter mean cyanobacteria chlorophyll *a* ranged from 1 – 4 μg l^{-1}, controlled largely by nutrient availability (Figure 5.8b).

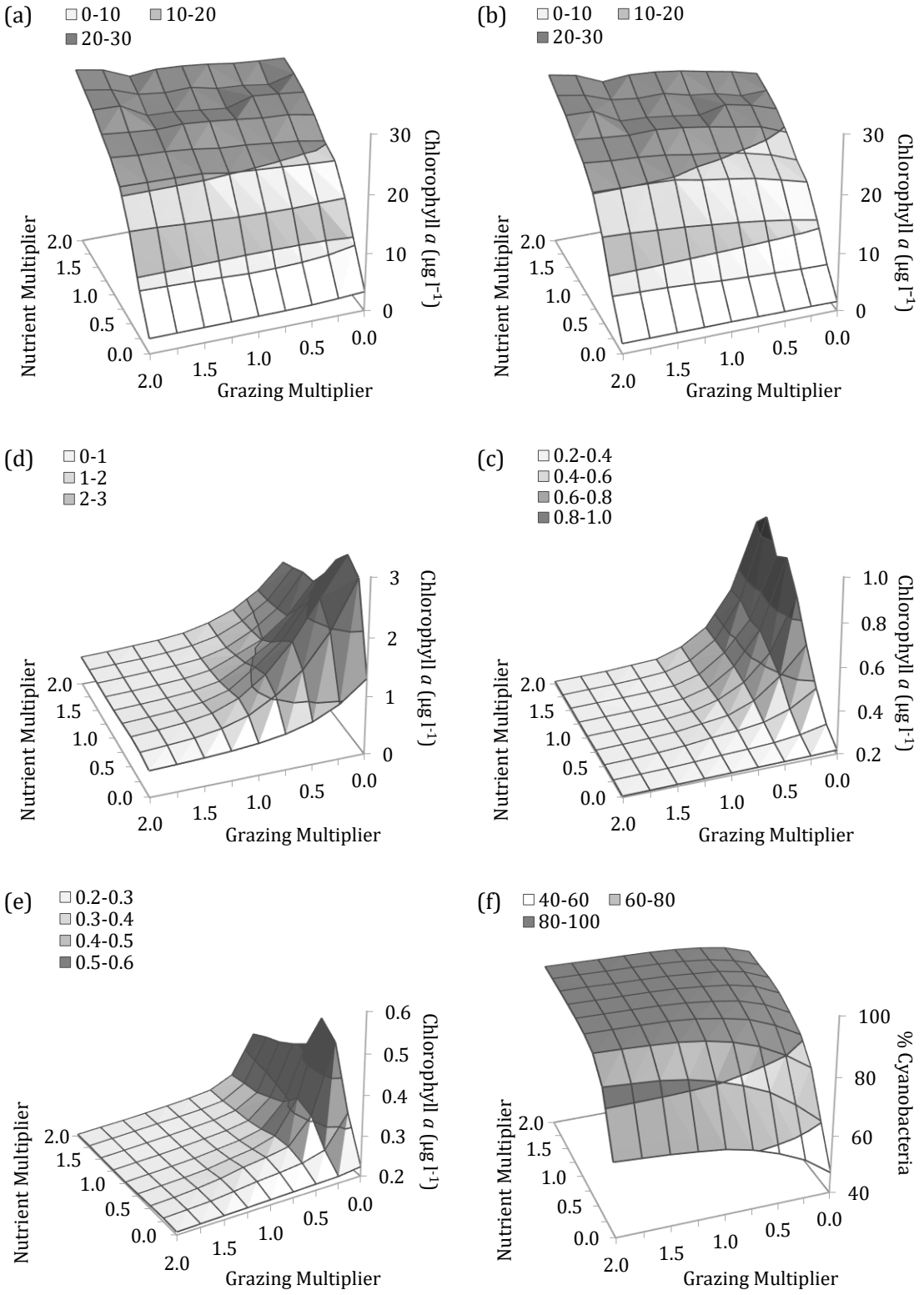

Figure 5.7: Autumn (September to November) mean chlorophyll *a* and percentage cyanobacteria abundance results, derived from 81 different scenarios of nutrient load and grazing rate: (a) total phytoplankton chlorophyll *a*, (b) cyanobacteria chlorophyll *a*, (c) diatom chlorophyll *a*, (d) flagellate chlorophyll *a*, (e) green algae chlorophyll *a*, and (f) percentage of total phytoplankton biomass accounted for by cyanobacteria.

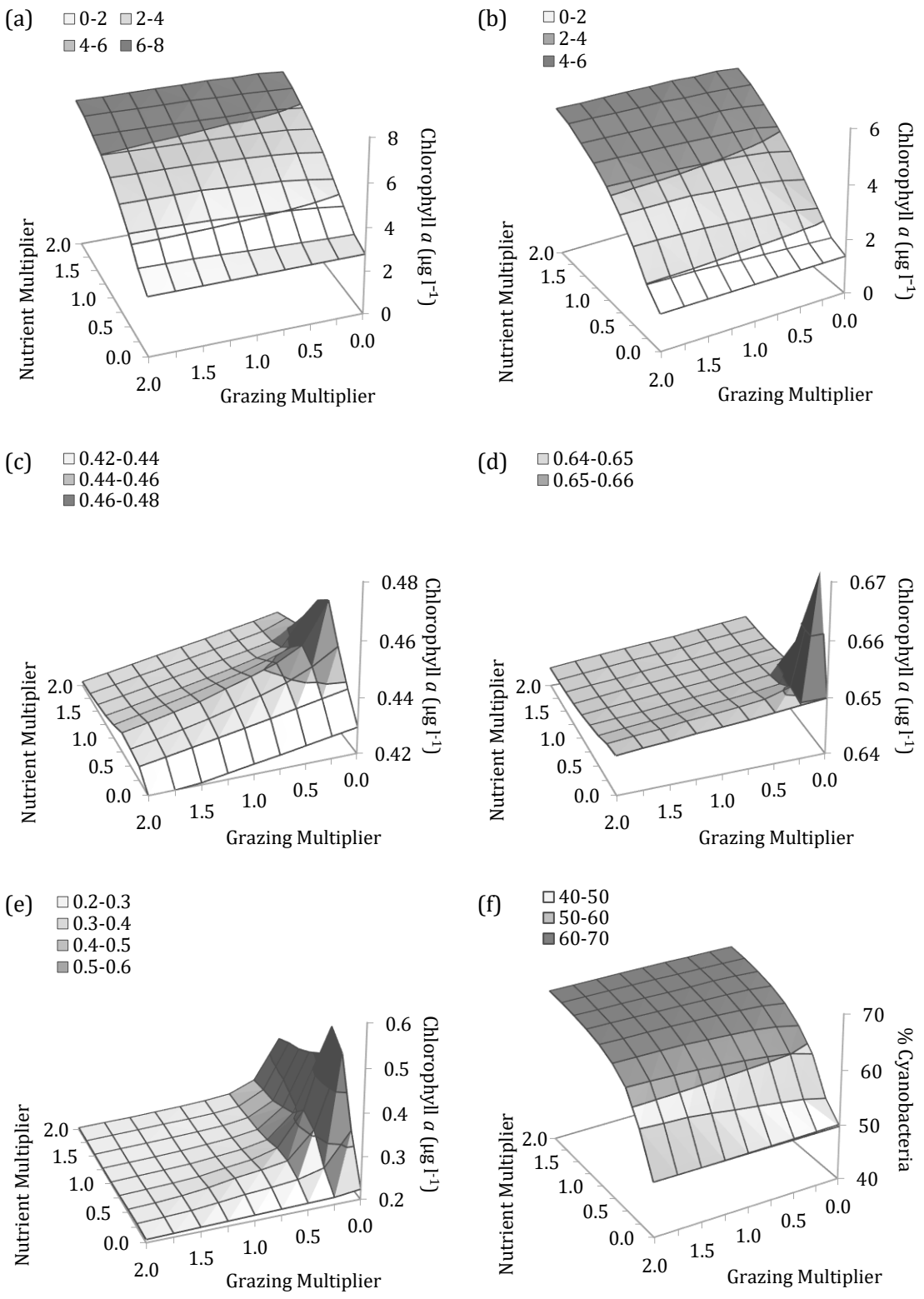

Figure 5.8: Winter (January to February, December) mean chlorophyll *a* and percentage cyanobacteria abundance results, derived from 81 model scenarios of nutrient load and grazing rate: (a) total phytoplankton chlorophyll *a*, (b) cyanobacteria chlorophyll *a*, (c) diatom chlorophyll *a*, (d) flagellate chlorophyll *a*, (e) green algae chlorophyll *a*, and (f) percentage of total phytoplankton biomass accounted for by cyanobacteria.

5.3.3 Impact of air temperature increase on the combined effects of nutrient load and grazing rate change

The results of the general linear models used to determine the effect of air temperature and its interactions with nutrient load and grazing rate on phytoplankton biomass are summarised in Table 5.3. Air temperature did not have a significant effect on annual mean phytoplankton biomass (Figure 5.9a-c). However, the air temperature-nutrient load interaction had a significant effect on the annual means of total chlorophyll *a* and non-cyanobacteria chlorophyll *a*. As temperature increased, the sensitivity of phytoplankton to nutrient variation diminished. Accordingly, total and non-cyanobacteria phytoplankton biomass decreased with increasing annual mean air temperature. At the seasonal scale, air temperature only had a direct impact on winter mean total chlorophyll *a* (Figure 5.9d), winter mean cyanobacteria chlorophyll *a* (Figure 5.9f) and summer mean non-cyanobacteria chlorophyll *a* (Figure 5.9g). Air temperature was close to being a significant explanatory variable for winter mean non-cyanobacteria chlorophyll *a* variation ($p = 0.05$, Figure 5.9i). Indirectly, air temperature impacted on the springtime cyanobacteria biomass by increasing responsiveness to nutrient loading (Figure 5.9e), and summer non-cyanobacteria phytoplankton biomass by dampening the responsiveness to nutrient load variability (Figure 5.9g). Significant interaction between air temperature and grazing rate was only determined for autumn mean non-cyanobacterial chlorophyll *a*. The effect of grazing was generally lower for simulations run above baseline temperatures and so there was a slight decreasing trend in autumn non-cyanobacteria biomass as temperature increased (Figure 5.9h).

Table 5.3: Results of the general linear models used to investigate the effect of air temperature, air temperature-nutrient load interaction and air temperature-grazing rate interaction on chlorophyll *a* concentration. Statistically significant results are denoted by bold, underscored text ($p < 0.05$).

Response Variable	Season	Air Temperature				Air Temperature – Nutrient Load				Air Temperature-Grazing Rate			
		Est.	St. Err.	*t*	*p*	Est.	St. Err.	*t*	*p*	Est.	St. Err.	*t*	*p*
Total Phytoplankton	Annual	1.32	2.72	0.49	0.63	-7.77	2.28	-3.40	**<0.001**	1.42	2.28	0.62	0.53
	Spring	0.42	0.73	0.57	0.57	-0.66	0.61	-1.08	0.28	-0.20	0.61	-0.32	0.75
	Summer	-0.25	0.52	-0.47	0.63	-0.80	0.44	-1.82	0.07	0.55	0.44	1.25	0.21
	Autumn	-0.05	8.81	-0.01	0.99	-13.05	7.40	-1.76	0.08	-1.14	7.40	-0.15	0.88
	Winter	3.65	1.50	2.44	**0.01**	1.86	1.26	1.48	0.14	-0.46	1.26	-0.37	0.71
Cyanobacteria	Annual	0.25	0.62	0.40	0.69	-0.30	0.52	-0.57	0.57	0.45	0.52	0.86	0.39
	Spring	0.03	0.07	0.52	0.60	0.23	0.06	4.09	**<0.001**	0.08	0.06	1.44	0.15
	Summer	0.06	0.24	0.23	0.82	-0.15	0.20	-0.73	0.47	0.18	0.20	0.86	0.39
	Autumn	3.83	7.90	0.49	0.63	-12.29	6.64	-1.85	0.06	-3.01	6.64	-0.45	0.65
	Winter	0.84	0.36	2.32	**0.02**	0.45	0.30	1.48	0.14	-0.10	0.30	-0.33	0.74
Non-Cyanobacteria	Annual	-0.15	0.15	-1.05	0.29	-0.43	0.12	-3.48	**<0.001**	0.02	0.12	0.19	085
	Spring	0.12	0.32	0.38	0.71	-0.48	0.27	-1.81	0.07	-0.15	0.27	-0.56	0.58
	Summer	0.02	0.01	2.49	**0.01**	0.00	0.01	0.68	0.50	0.01	0.01	1.73	0.08
	Autumn	0.05	0.03	1.72	0.09	-0.02	0.02	-0.77	0.44	0.05	0.02	2.02	**0.04**
	Winter	0.00	0.00	-1.95	0.05	0.00	0.00	0.74	0.46	0.00	0.00	0.22	0.83

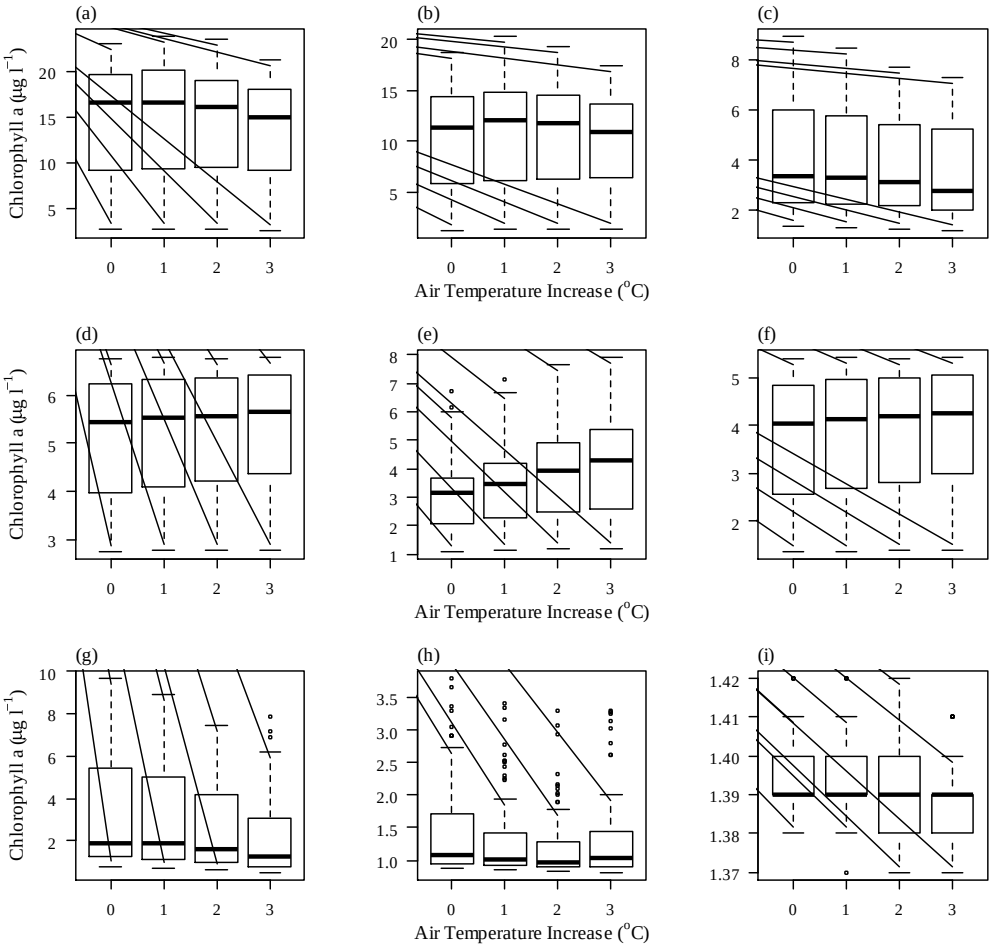

Figure 5.9: Box plots of mean chlorophyll *a* concentration derived from PROTECH simulations of phytoplankton community at four different annual mean air temperatures. All metrics displayed were significantly impacted by annual mean air temperature increase. The black line indicates the median, the 25th and 75th percentiles are represented by the box, whiskers show the 10 – 90 % percentiles and outliers are represented as circles. (a) Annual total, (b) annual cyanobacteria, (c) annual non-cyanobacteria, (d) winter total, (e) spring cyanobacteria, (f) winter cyanobacteria, (g) summer non-cyanobacteria, (h) autumn non-cyanobacteria, and (i) winter non-cyanobacteria.

5.3.4 Long-term projections of phytoplankton community

To explore potential lake recovery trajectories, the phytoplankton community in Esthwaite Water was modelled over a 30-year period under different scenarios of internal phosphorus load decay and air temperature increase. Long-term reduction of internal phosphorus load is projected to have a clear impact on phytoplankton biomass. Under conditions of no air temperature change (i.e. 2014 air temperature measurements were repeated year-on-year), annual mean modelled chlorophyll *a* concentrations declined over time with decreasing internal load (Figure 5.10a). In comparison, modelled chlorophyll *a* was not projected to decrease over time when the internal phosphorus load

was maintained at year one (2014) levels throughout. Chlorophyll *a* concentrations under higher decay rate scenarios (> 10 % reduction in load per annum) decreased to a plateau of *c*. 13 µg l^{-1} within the 30-year modelling period. However, none of the internal phosphorus load decay scenarios were sufficient to reduce the annual mean modelled chlorophyll *a* concentration to the level required to achieve EU WFD Good Ecological Status.

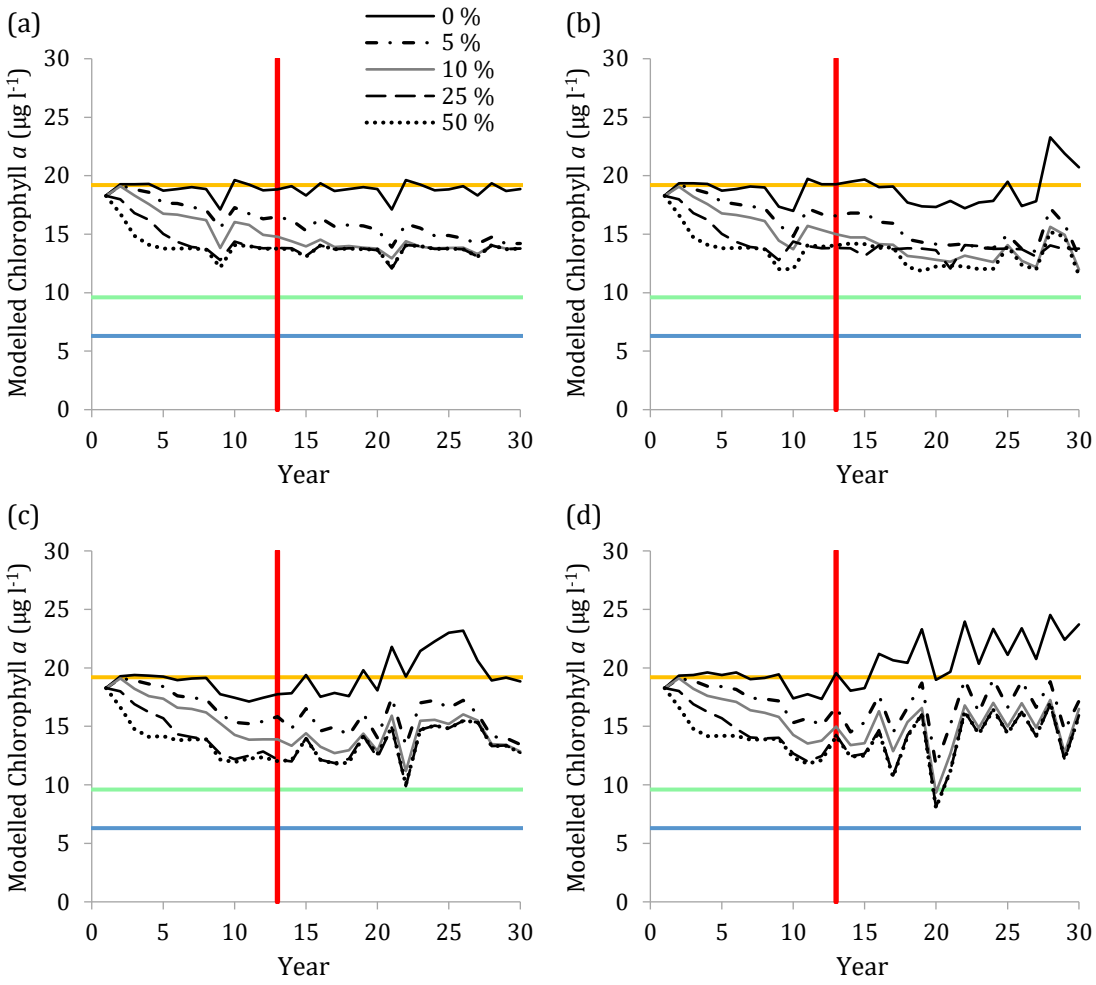

Figure 5.10: Time series of annual mean modelled chlorophyll *a* concentration. Legend denotes the annual reduction of internal phosphorus load. Annual mean air temperature increase between the first year of the simulation and final year of the simulation: (a) 0 °C, (b) 1 °C, (c) 2 °C, and (d) 3 °C. EU WFD chlorophyll *a* boundaries specific to Esthwaite Water: blue = high-good boundary (6.3 µg l^{-1}), green = good-moderate boundary (9.6 µg l^{-1}), and yellow = moderate-poor boundary (19.2 µg l^{-1}). The EU WFD target for achieving Good Ecological Status is indicated by the red line at year 13 (2027).

When internal phosphorus load decay was combined with air temperature increase the projected chlorophyll *a* concentration initially followed a similar trajectory to those described above (Figures 5.10b-d). Comparisons between simulations of the same internal phosphorus load decay rate but differing rates of air temperature increase showed little difference in the phytoplankton biomass produced during the first decade of the model run. Phytoplankton biomass decreased steadily with decreasing internal load, again stabilising at *c*. 13 µg l^{-1} for scenarios with an internal phosphorus load decay rate > 10 % per annum. However, at a specific point during each of the simulations there appeared to be a shift in phytoplankton biomass trajectory. Modelled chlorophyll *a* concentrations began to increase gradually, regardless of whether they appeared to have already stabilised. In the plus 3 °C air temperature scenarios, the shift occurred in year 16 after an increase of 1.55 °C in annual mean air temperature. In the plus 2 °C scenarios the shift occurred in year 21, corresponding to a 1.38 °C increase in annual mean air temperature over time. Finally, the shift occurred in year 28 in the 1 °C increase scenarios, equivalent to a 0.93 °C increase in annual mean air temperature since year one. In projections where the internal phosphorus load did not decay, the projected annual mean chlorophyll *a* concentration increased beyond the EU WFD boundary from Moderate Ecological Status to Poor Ecological Status.

Long-term trends in cyanobacteria biomass were similar to those of total phytoplankton biomass. Cyanobacteria chlorophyll *a* concentration initially decreased with decaying internal load to plateau at *c*. 8 µg l^{-1}. The upward shift in cyanobacteria biomass occurred at the same air temperature change thresholds as for total phytoplankton biomass, although the increase in cyanobacteria biomass was noticeably steeper (Figure 5.11). Non-cyanobacteria phytoplankton biomass responded similarly to decreasing internal load, plateauing at *c*. 3 µg l^{-1}. Unlike cyanobacteria, the biomass of non-cyanobacteria phytoplankton began to decline sharply at threshold air temperatures (Figure 5.12). The contrasting responses of cyanobacteria and non-cyanobacteria phytoplankton to air temperature increase account for the dampened response of total phytoplankton biomass. Consequently, the percentage of total phytoplankton biomass accounted for by cyanobacteria increased over time in all combinations of internal phosphorus load decay and air temperature increase that were tested. Only when internal phosphorus load declined and annual mean air temperature remained constant was there a slight decrease in the percentage abundance of cyanobacteria over the modelling period (Figure 5.13). The WHO threshold of 10 µg l^{-1} cyanobacteria chlorophyll *a* was exceeded less frequently

over time as internal phosphorus load decreased. However, the effect of internal phosphorus load decay was counteracted by increasing air temperature (Figure 5.14).

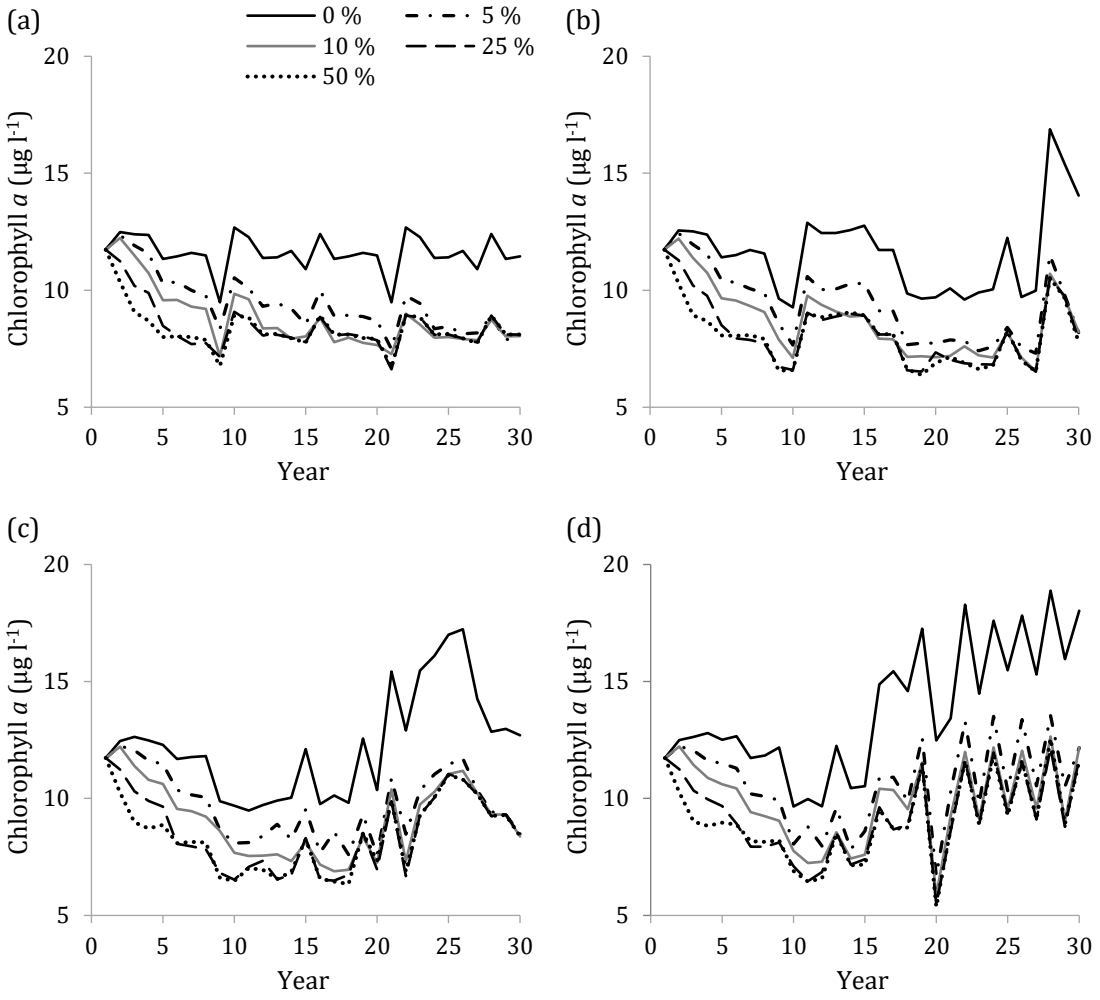

Figure 5.11: Time series of cyanobacteria chlorophyll *a* concentration. Legend denotes the annual reduction of internal phosphorus load. Annual mean air temperature increase between the first year of the simulation and final year of the simulation: (a) 0 °C, (b) 1 °C, (c) 2 °C, and (d) 3 °C.

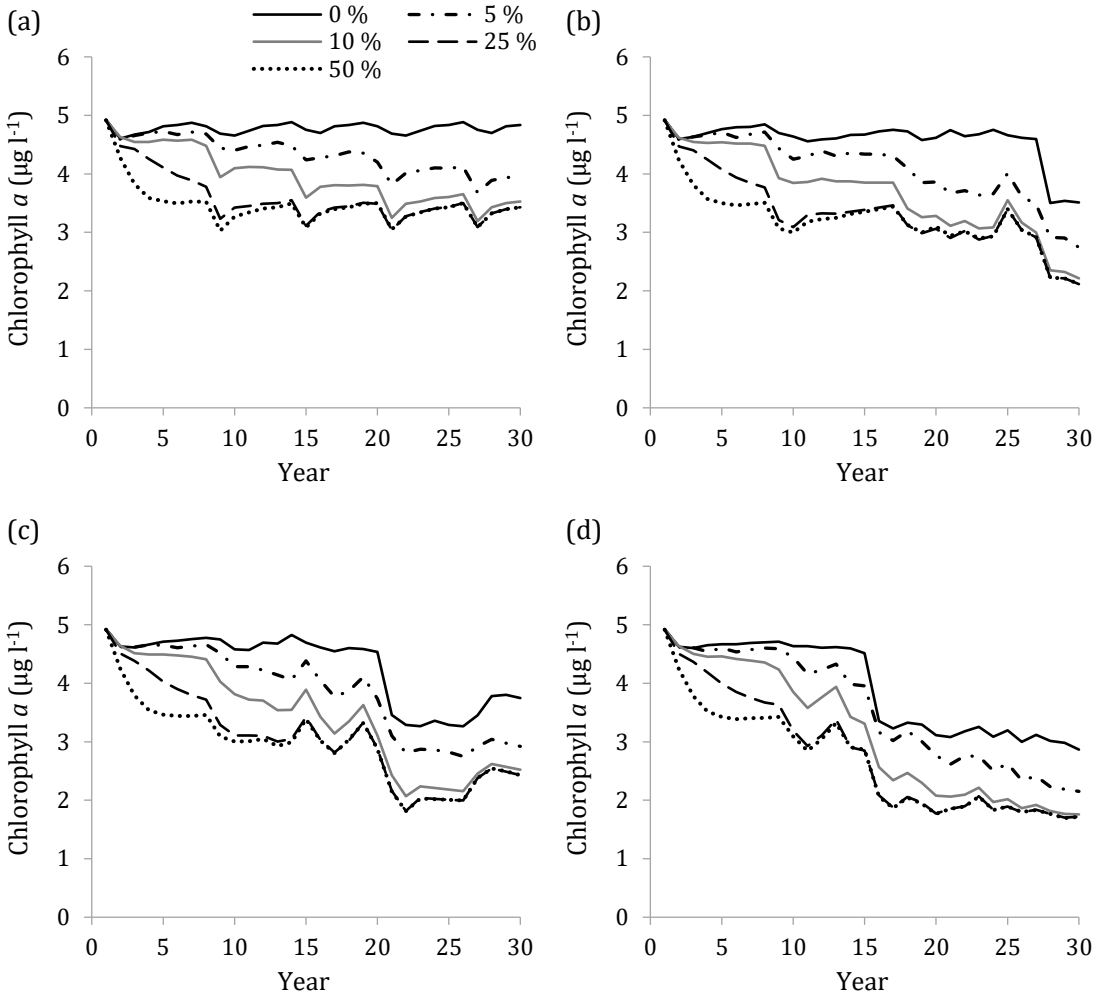

Figure 5.12: Time series of annual mean non-cyanobacteria chlorophyll *a* concentration. Legend denotes the annual reduction of internal phosphorus load. Annual mean air temperature increase between the first year of the simulation and final year of the simulation: (a) 0 °C, (b) 1 °C, (c) 2 °C, and (d) 3 °C.

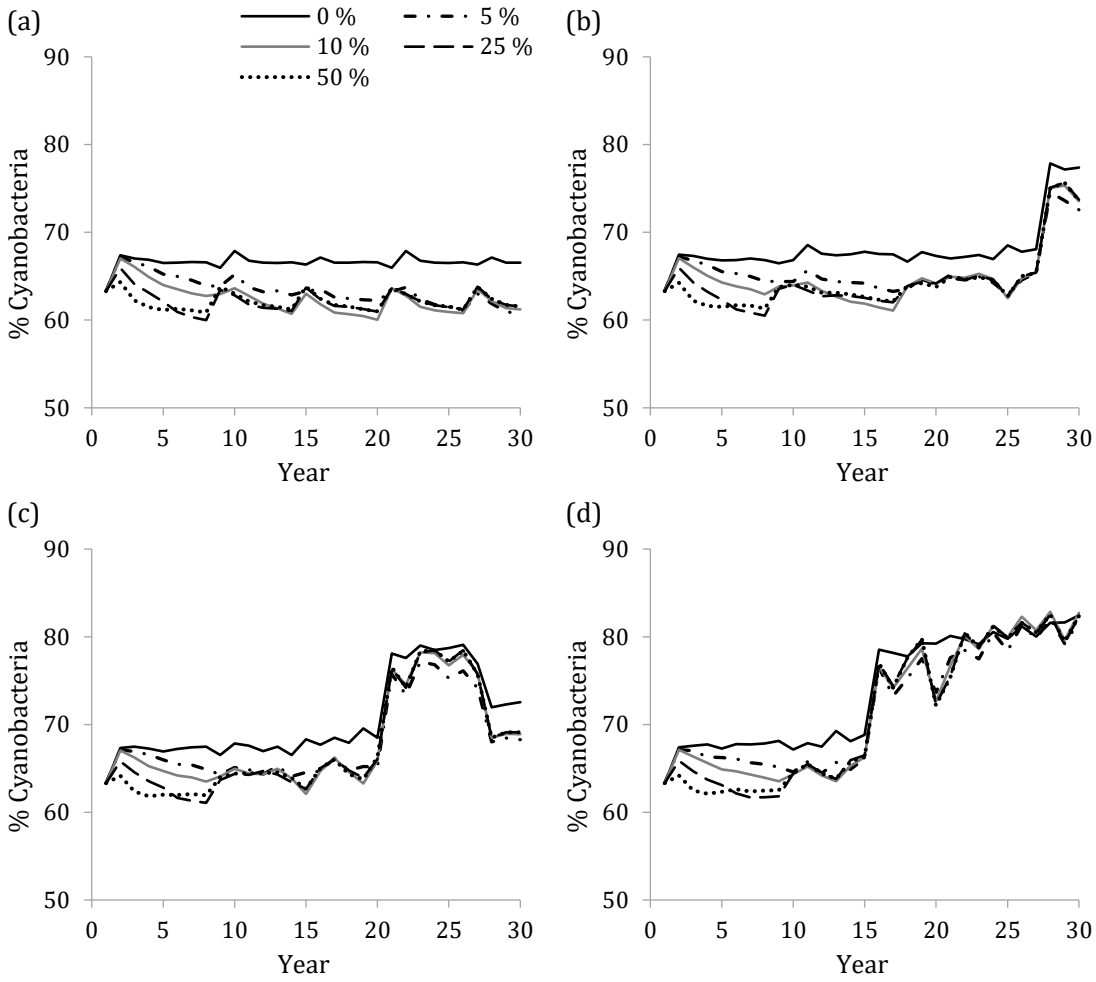

Figure 5.13: Time series of the annual mean percentage of total phytoplankton biomass accounted for by cyanobacteria. Legend denotes the annual reduction of internal phosphorus load. Annual mean air temperature increase between the first year of the simulation and final year of the simulation: (a) 0 °C, (b) 1 °C, (c) 2 °C, and (d) 3 °C.

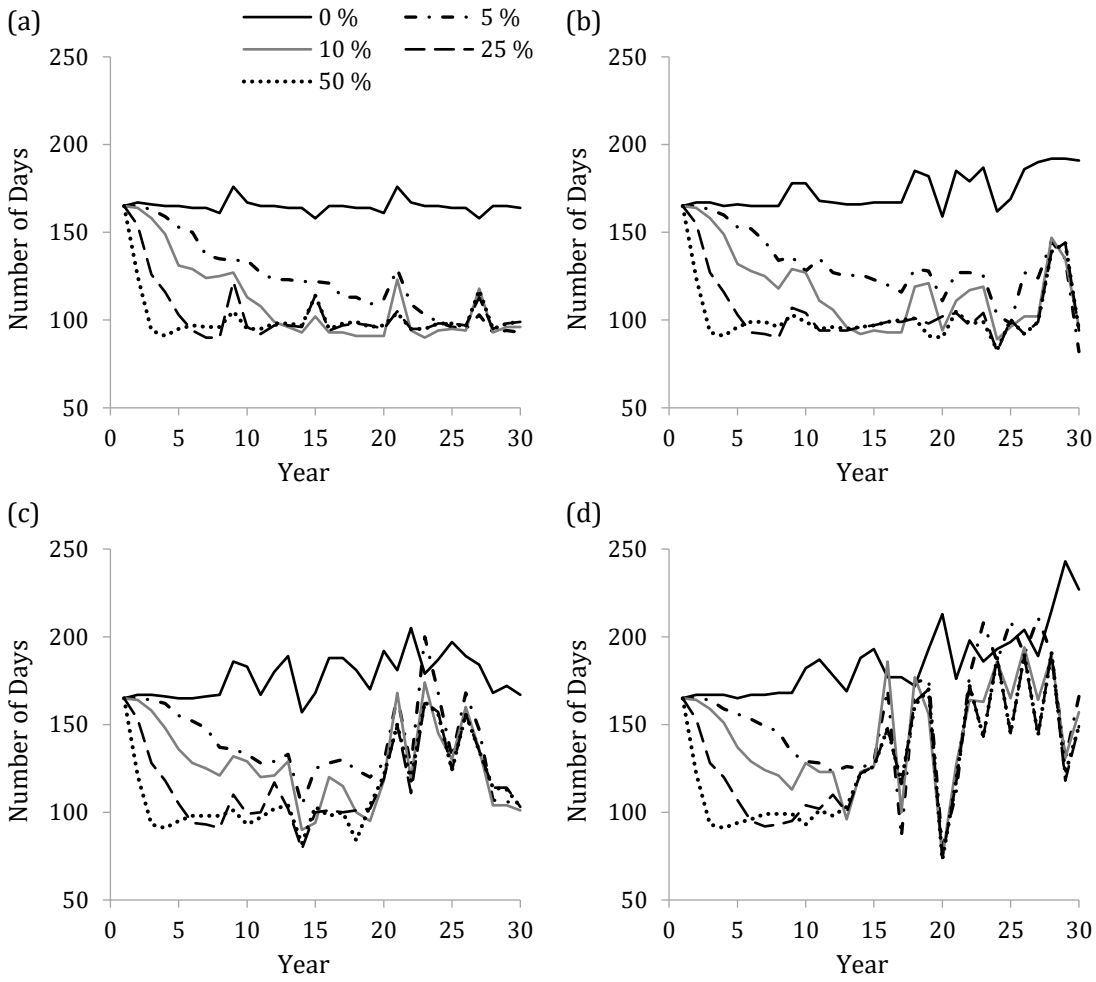

Figure 5.14: Time series of the number of days per year that the WHO cyanobacteria concentration threshold of 10 µg l^{-1} is exceeded. Legend denotes the annual reduction of internal phosphorus load. Annual mean air temperature increase between the first year of the simulation and final year of the simulation: (a) 0 °C, (b) 1 °C, (c) 2 °C, and (d) 3 °C.

5.4 Discussion

5.4.1 Top-down versus bottom-up control of the phytoplankton community

One of the main objectives of this study was to gain an understanding of how changes in top-down (*Daphnia* grazing rate) and bottom-up (nutrient availability) forces might influence the development of phytoplankton in a freshwater lake. Factorial design experiments such as this one, in which resource availability and predation pressure are manipulated, provide a strong test of the individual and combined effects of these forces (Hunter 2001). With the exception of nutrient load and grazing rate, all of the data used to drive the model remained unchanged. So, any variation in the output data between the tested scenarios could solely be attributed to these factors.

The highest annual mean total chlorophyll *a* concentration was recorded for the scenario which combined highest nutrient load with lowest grazing rate. However, this pattern of annual mean chlorophyll *a* response to nutrient load and grazing rate variation was not observed for all four of the phytoplankton groups considered in this study. While an increase in nutrient load did favour growth in all of the genera simulated, the response to grazing rate variation was not consistent. Contrary to the response of the three other phytoplankton groups, cyanobacteria biomass increased with increasing grazing rate. It is generally considered that *Daphnia* avoid consuming cyanobacteria (Ger *et al.* 2014; Visser *et al.* 2005). For that reason, calculations of grazing losses on phytoplankton biomass were not applied to any of the cyanobacteria included in the model. Consequently, as the populations of green algae, diatoms and flagellates were reduced by grazing, competition for nutrients or other resources, would also have decreased and cyanobacteria proliferated as a result. The contrasting response patterns of cyanobacteria to the other taxonomic groups had the effect of dampening the impact of grazing on annual mean total chlorophyll *a* levels.

If the annual mean value of total chlorophyll *a* alone is used to evaluate the relative importance of phytoplankton community control mechanisms, then it would appear that bottom-up regulation by nutrient availability is of greater importance than top-down control by grazing. The impact that individual groups had on the response of total biomass to the variation of top-down and bottom-up pressures was more evident when the seasonal trends were examined. Seasonal mean chlorophyll *a* response surfaces of the individual taxonomic groups did not deviate much from those of annual mean chlorophyll *a.* However, the response of total phytoplankton biomass to nutrient load and grazing rate did vary between seasons; it typically reflected the response pattern of whichever taxonomic group was most dominant at the time.

Diatoms, specifically *Asterionella*, dominated the spring phytoplankton maximum in the baseline simulation. *Asterionella* was able to exploit this early growing period as they have a higher potential in the model, as they do in reality, to grow at lower levels of insolation than other phytoplankton taxa (Elliott 2010; Reynolds *et al.* 2002). This dominance was maintained for almost all combinations of grazing rate and nutrient load tested. Diatoms exhibited a strong response to both the top-down and bottom-up pressures, evident also in the spring mean chlorophyll *a* response pattern. Only when the grazing rate was very high did the un-grazed cyanobacteria take over as the most abundant group. Although total phytoplankton biomass was at its highest in the summer, diatom biomass was

considerably reduced compared to the springtime. Cyanobacteria succeeded as the most dominant group, in some scenarios accounting for up to 97 % of the total biomass. Cyanobacteria are often the dominant taxa in eutrophic warm waters on account of their ecophysiological advantages (Dokulil & Teubner 2000; Jöhnk *et al.* 2008; Reynolds 2006; Wagner & Adrian 2009). This bimodal pattern of seasonal succession is frequently observed in temperate eutrophic lakes (Reynolds 1984a; Sommer *et al.* 1986), including in previous studies of Esthwaite Water (George *et al.* 1990; Talling & Heaney 1988). The late spring diatom crash can be attributed to several factors: silica limitation; acceleration of zooplankton recruitment, and hence grazing pressure, as temperatures increase; and the sedimentation of cells as the water column stabilises (Sommer *et al.* 1986).

Nutrient availability clearly exerted greater control over summer cyanobacteria biomass, and therefore summer total biomass, than grazing pressure. Even so, it appears that the two factors had a synergistic effect. The response of cyanobacteria to each step-wise change in the nutrient load was enhanced as the grazing rate increased, possibly because a higher rate of grazing by *Daphnia* diminished the stock of edible phytoplankton and consequently reduced competition for the increased nutrient supply. Green algae, flagellates and diatoms each exhibited a strong reaction to grazing rate variability during the summer but as their combined contribution to the overall biomass was relatively small this effect was masked somewhat in the response surface of summer mean total chlorophyll *a*. The impact of nutrient availability on these genera was negligible, especially under conditions of high grazing rate. According to the Plankton Ecology Group (PEG) model of Sommer *et al*. (1986), zooplankton population size is resource limited during prolonged blooms of inedible algae. Presumably, the lack of response of these minor taxa to nutrient load variability was because any new edible phytoplankton biomass was rapidly lost due to grazing by the *Daphnia*.

In the autumn total phytoplankton biomass remained high and cyanobacteria persisted as the dominant taxa. As in the summer, the minor taxonomic groups exhibited a stronger response to changes in grazing rate than nutrient load. Whereas cyanobacteria biomass had not declined since the summer, flagellates, green algae and diatom biomass decreased appreciably. As such, they had no discernible impact on the response of total phytoplankton biomass to variations in the top-down and bottom-up pressures, which was entirely influenced by the response of the dominant cyanobacteria. The biomass of all taxa included in the model were at their lowest in winter. The winter minimum was

undoubtedly due to light limitation rather than the inhibition of phytoplankton growth by either of the variables being investigated here (Talling 1971).

In agreement with field-based observations (Carpenter *et al.* 2001; Hansson 1992; Jeppesen *et al.* 2003) and empirical models (Kerimoglu *et al.* 2013), the results of this study demonstrate that phytoplankton biomass and community structure is regulated by both top-down and bottom-up processes, and that these processes are not independent of one another. Nutrient availability, and therefore potential limitation, is intrinsically linked to the grazing activity of zooplankton, and vice-versa. Moreover, sensitivity to these pressures varies between phytoplankton type. Therefore, the relative importance of top-down and bottom-up effects in regulating phytoplankton biomass varies throughout the seasons with successional changes in planktonic community structure.

These findings pose some implications for the management and regulation of eutrophic lakes. Annual mean values of phytoplankton chlorophyll *a* are often used to determine the overall quality of a waterbody. Following this principal, the simulations of phytoplankton communities presented here imply that water quality deteriorates as nutrient load increases and *Daphnia* grazing rate decreases. This suggests that water quality improvements could be achieved by: (i) reducing the availability of nutrients for uptake by phytoplankton, (ii) instigating a trophic cascade to increase *Daphnia* grazing pressure on phytoplankton, or (iii) a combination of the two remediation strategies. However, while nutrient load limitation had a uniform effect across all of the phytoplankton taxa included in the simulations, the response to grazing rate increase was not consistent. Unlike the other phytoplankton groups, cyanobacteria proliferated under conditions of increased *Daphnia* grazing rate. Therefore, remediation of eutrophic lakes by biomanipulation may be effective at reducing the total phytoplankton chlorophyll *a* concentration to comply with legislative water quality criteria (e.g. EU WFD), but it could also compromise the provision of some ecosystem services by promoting the growth of nuisance cyanobacteria.

5.4.2 Impact of air temperature increase on the top-down and bottom-up control of the phytoplankton community

A growing concern in limnology over the last decade has been to understand how climate change might affect the structure and function of freshwater systems. Of particular interest is the impact on mechanisms which regulate the phenology, abundance and composition of phytoplankton communities. To this end, PROTECH was used to

investigate the combined effects of air temperature, grazing rate and nutrient load variation on the phytoplankton community of Esthwaite Water.

The response patterns of annual mean chlorophyll *a* to air temperature increase were clearly influenced by the effect of warming on the summer phytoplankton community. Although the proportion of summer phytoplankton biomass accounted for by cyanobacteria did increase with increasing temperature, it was not due to the proliferation of cyanobacteria as hypothesised. Rather, cyanobacteria biomass remained relatively unchanged with warming while the biomass of non-cyanobacteria phytoplankton declined. This effect was an indirect consequence of warming due to the diminished sensitivity of non-cyanobacteria taxa to nutrient load variation as temperature increased. As cyanobacteria biomass did not increase, the decline in non-cyanobacteria biomass cannot be attributed to increased competition for resources. In contrast to cyanobacteria, most of the other phytoplankton types included in the model are unable to control their vertical position. They are reliant on water column turbulence to remain suspended in the euphotic zone (Huisman *et al.* 2002; Reynolds 1983). Prolonged periods of thermal stratification resulting from climate warming are likely to have brought about considerable sinking losses of non-cyanobacteria biomass (Reynolds 2006), thus reducing their responsiveness to nutrient load variation during the summer.

Surprisingly, the ecophysiological characteristics that are generally associated with cyanobacteria dominance during lake stratification do not appear to have significantly enhanced summer growth in the simulations with increased air temperature. This would suggest that phytoplankton growth in Esthwaite Water was limited by phosphorus availability. Under such conditions, the ability to fix atmospheric nitrogen and control vertical water column position would be of little advantage to cyanobacteria. The decline in non-cyanobacteria phytoplankton biomass at increased air temperature would have made more phosphorus available for uptake by cyanobacteria. However, given the dominance of cyanobacteria during the summer this increase in phosphorus availability would have been negligible in terms of its impact on cyanobacteria biomass. Interestingly, previous applications of PROTECH to Esthwaite Water predicted enhanced cyanobacteria abundance with increasing water temperature, particularly during the summer (Elliott 2010). These earlier simulations were driven using measurements of inflow nutrient concentration collected during a period of higher external phosphorus loading. The contrasting results of the two modelling investigations support the theory that predicted

climate change impacts on phytoplankton communities could be minimised by reducing nutrient inputs (Moss *et al.* 2011; Paerl *et al.* 2011).

Although there was not a significant change in cyanobacteria biomass in response to air temperature increase during the main summer growing season, there were significant increases during other periods of the year. The sensitivity of spring mean cyanobacteria chlorophyll *a* concentration to nutrient load variability was heightened with air temperature increase. In Esthwaite Water cyanobacteria development in spring is usually limited by low insolation and deeper mixing rather than by nutrient availability (George 2012; George *et al.* 1990). Earlier onset of stratification, induced by springtime warming, would have produced favourable water column conditions for nutrient assimilation by cyanobacteria (Huisman *et al.* 2004; Wagner & Adrian 2009), hence the spring biomass increase. Winter cyanobacteria biomass also increased significantly with increasing annual mean air temperature, although as a direct temperature effect rather than through the interaction of air temperature with either nutrient availability or grazing rate. PROTECH does not apply different growth rate equations to each of the phytoplankton included in the model based on their thermal tolerances; all growth is determined by the defined morphological variables (Table 5.1). In PROTECH, as in real phytoplankton populations, large volume phytoplankton (e.g. cyanobacteria) grow relatively slowly compared to smaller volume phytoplankton (Reynolds 1984b, 2006). Significantly, this relative difference in growth rate diminishes with increasing temperature. As a result, winter cyanobacteria biomass was enhanced with increasing annual mean air temperature, whereas the direct effect of warming on non-cyanobacteria phytoplankton was not sufficient to render a significant increase.

The effect of *Daphnia* grazing rate on autumn mean non-cyanobacteria chlorophyll *a* was significantly diminished as air temperature increased. In PROTECH *Daphnia* grazing rate is dependent on the availability of edible phytoplankton. It appears that food limitation due to the decline of non-cyanobacteria phytoplankton biomass during the summer had a negative bottom-up effect on autumnal grazing rate with air temperature increase.

5.4.3 Long-term projections of the phytoplankton community

Recent restoration efforts at Esthwaite Water have focussed mainly on reducing the external phosphorus load. As the external load has declined over the last decade there has been a gradual shift from annual net phosphorus uptake by lake sediments to an annual net release of the accumulated phosphorus back into the water column (refer to Chapter

Three). Attempts to improve the ecological quality of many eutrophic lakes have been hindered by this process as internal loading provides the ecosystem with a degree of resilience to the loss of other phosphorus sources (Jeppesen *et al.* 1991; Søndergaard *et al.* 2003). Over time the magnitude of the internal phosphorus load will decrease until a new equilibrium is reached between the sediment and water column (Marsden 1989). It is impossible to accurately predict the duration of sediment phosphorus flux re-equilibration or the long-term influence of the internal load on phytoplankton community structure and biomass. In this study multi-year phytoplankton community simulations were conducted under a range of sediment phosphorus flux decay rates to assess the internal load trajectory required if Esthwaite Water is to meet EU WFD chlorophyll *a* objectives by 2027.

Under current climatic conditions, it appears that the EU WFD chlorophyll *a* targets will not be met in Esthwaite Water within the required timeframe, regardless of the decay rate applied to the sediment phosphorus flux. Although chlorophyll *a* concentrations did decrease initially, they plateaued at *c*. 13 μg l^{-1}, coincident with the stabilisation of the sediment phosphorus flux. Given that all other driving variables remained constant it is unsurprising that the simulated trajectory for chlorophyll *a* concentration mimics that of internal phosphorus load. The annual net internal load was predicted to stabilise at *c*. 110 kg y^{-1}, although the stabilised load was only reached within the 30-year modelling period when the sediment phosphorus flux decay rate was greater than 10 % per annum. The internal load was predicted to stabilise at this value rather than continue to decay exponentially to almost zero because the hypolimnion sediment phosphorus fluxes between May and September, when the lake is typically stratified (George & Hewitt 1999), were maintained at year one (2014) rates throughout the projection. Sediment phosphorus flux to the hypolimnion was protected from decay during this period each year to account for the potential effect of physiological processes on lake recovery prospects.

Currently, much of the sediment phosphorus flux during stratification is probably caused by the release of phosphate bound to iron oxide at the anaerobic sediment-surface into an oxygen-depleted hypolimnion (refer to Chapter Four). In PROTECH, a portion of this load is transferred to the euphotic zone by intermittent vertical extension of the mixed surface layer or by the eventual turn-over of the lake in autumn, the timing and extent of which are determined as a function of heat fluxes and wind-induced turbulence (Elliott & Thackeray 2004). Based on the evidence from remediation attempts in other stratifying

lakes, historical sediment oxygen demand and the ongoing sedimentation and decomposition of organic matter is likely to continue exceeding the hypolimnion oxygen reserve during summer stratification. Consequently, the release of reductant-soluble phosphorus has the potential to promote phytoplankton growth for many years, despite the external nutrient load reductions. This was also the case in Loch Leven, Scotland. Like Esthwaite Water, Loch Leven received elevated phosphorus inputs from its catchment over many decades, which resulted in a deterioration of water quality (May *et al.* 2012). Although the external phosphorus load was reduced by 60 % between the 1970s and 1990s water quality improvements were not observed until 20 years later. The delay in recovery has been ascribed to the release of sediment phosphorus, with the anoxic dissolution of reductant-soluble phosphorus forms during stratification identified as a significant portion of the load (Spears *et al.* 2012).

In addition, hydraulic retention time in Esthwaite Water tends to be longer during the summer (George *et al.* 2007), so the rate of phosphorus removal from the system by flushing is likely to be low at this time of year. In particular, flushing of phosphorus-rich water from the hypolimnion may be negligible during stratification if the kinetic energy input of the hydraulic load is not sufficient to penetrate the thermocline. Rather than be flushed from the lake, it is reasonable to assume that much of the phosphorus released during stratification will be recycled back into the sediment by the precipitation of iron-phosphates when the hypolimnion is re-oxygenated. As a result, the release-sensitive phosphorus pool in sediment will be sustained and will continue to be released to the overlying water column during future periods of anoxia.

By exempting the May to September sediment phosphorus fluxes from decay the seasonality of some of the most important processes that control sediment phosphorus mobilisation and retention were represented in the models. To ignore them would likely have resulted in an overestimation of the water quality improvements that could be expected in Esthwaite Water over the coming decades. However, the projections of sediment phosphorus flux used in these simulations do not take into account the numerous other processes that contribute to a decrease in the rate of sediment phosphorus release. For example, burial of the enriched sediment by material with a lower phosphorus concentration or the conversion of sediment phosphorus to refractory forms, such as apatite, which have low solubility (Marsden 1989; Reitzel *et al.* 2012). The long-term simulations of phytoplankton community presented here can, therefore, be considered as worst-case scenarios for the recovery trajectory of Esthwaite Water.

As hypothesised, air temperature increase did counteract the effect of internal phosphorus load decay in the multi-year simulations of phytoplankton community. Annual means of total phytoplankton chlorophyll *a* and cyanobacteria chlorophyll *a* both increased with increasing air temperature. Based on the results of the single-year simulations of phytoplankton community, which saw a reduction in the annual mean concentration of total chlorophyll *a* and no significant change in cyanobacteria chlorophyll *a* with increasing air temperate, the prediction of long-term increase is perhaps surprising. The key to explaining this paradox could be the effect of warming on winter cyanobacteria populations. In the single-year simulations the winter mean cyanobacteria biomass increased significantly with warming. As winters became progressively milder throughout the long-term simulations, the survival rate and growth of overwintering cyanobacteria improved. Consequently, the cyanobacteria inoculum at the start of the main growing season increased year-on-year. So, while the winter increase was not large enough to manifest a significant increase at the annual scale, it could have been important in the implementation of long-term phytoplankton community change.

The simulated increase in relative abundance of cyanobacteria with warming is consistent with trends observed in long-term time series data from a number of lakes (Jacquet *et al.* 2005; Kosten *et al.* 2012; Posch *et al.* 2012; Wagner & Adrian 2011). The shift towards cyanobacteria dominance with increasing air or water temperature has been related to climate-induced changes in the thermal regime of a lake, rather than direct temperature effects (Wagner & Adrian 2009). As discussed previously, certain ecophysiological adaptions of cyanobacteria (e.g. buoyancy regulation, the ability to fix nitrogen) give them an advantage over other phytoplankton in the stable water column conditions that coincide with high temperatures (Carey *et al.* 2012; Paerl & Huisman 2008). However, statistical analysis of the long-term data is not sufficient by itself to confirm that cyanobacteria are already benefiting from warmer temperatures, or that they will continue to in the future. The factors that control phytoplankton community structure are complex and it can be difficult to disentangle the effects of climate change from the effects of other environmental variables. Process-based models, such as PROTECH, are useful in that simulation driving conditions can be controlled to test the effect of a specific variable (Elliott *et al.* 1999). The multi-year simulations presented here, during which air temperature increased while all other driving variables remained unchanged, provide strong evidence to support the theory that predicted warming will increase cyanobacteria abundance.

The predicted effect of climate warming on freshwater phytoplankton communities is likely to have a detrimental impact on the provision of many essential ecosystem services. WHO have recommended guideline values for cyanobacterial abundance in recreational waters, which are associated with the severity and probability of adverse health effects (Chorus & Bartram 1999; WHO 2003). In the long-term simulations, cyanobacteria abundance exceeded the moderate health risk threshold with increasing frequency as air temperature increased. This clearly demonstrates the consequences of warming on ecosystem services. In particular, the use of lakes for recreational activities and the urgent need for implementation of mitigation strategies to secure their longevity.

Although increasing air temperature promoted phytoplankton growth, the actual magnitude of biomass increase was greatly dependent upon the nutrient resource base available. The effect of air temperature increase on phytoplankton community was greatest in the scenarios that incorporated lower internal phosphorus load decay rate. On the other hand, the effect was greatly reduced under conditions of high internal load decay rate. These findings offer further support to the theory that in a future warmer climate, nutrient concentrations will need to be reduced significantly from present values if cyanobacteria dominance is to be controlled (Kosten *et al.* 2012; Moss *et al.* 2011; Trolle *et al.* 2011). In recent years, many lakes have already undergone substantial nutrient load reductions to combat the effects of eutrophication, including Esthwaite Water. In the face of climate warming the likelihood of such lakes achieving or maintaining favourable ecological and water quality conditions will be diminished (Bennion *et al.* 2011; Moss *et al.* 2011).

5.4.4 PROTECH limitations and development

The baseline model constructed for this study produced data that correlated more strongly to in-field measurements than in any of the other investigations recorded in PROTECH literature (Elliott 2010; Elliott *et al.* 2006; Elliott & Defew 2011; Elliott & Thackeray 2004; Lewis *et al.* 2002). The most notable difference between this investigation and those conducted previously is that sediment phosphorus flux was calculated and included as a driver in the simulations. Countless studies have highlighted the importance of sediment in the lake phosphorus cycle (Drake & Heaney 1987; Sas 1989; Søndergaard *et al.* 2003; Welch & Cooke 1995). Even so, previous PROTECH investigations have either omitted internal phosphorus load altogether or calibrated the input nutrient data to compensate for the difference between simulated and measured in-lake SRP

concentrations, attributing this missing load to sediment phosphorus release (Elliott 2010; Elliott & Defew 2011). However, the SRP concentration simulated by PROTECH for the surface 5 m of the water column is determined by a number of factors besides nutrient load, e.g. climatic conditions, algal community composition and *Daphnia* grazing rate. Deviation from in-field measurements cannot necessarily be ascribed solely to a deficiency in the amount of nutrients added to the model; other driving data may also be inaccurate. The mass balance calculation used here to quantify the internal phosphorus load is based on relatively high-resolution measurements of external phosphorus load and phosphorus export from the lake. Therefore, the temporal variation in sediment phosphorus flux is represented, including periods of phosphorus uptake, and an accurate estimate of the annual net internal phosphorus load produced. Correspondingly, a better overall validation was achieved here than in previous PROTECH investigations.

Esthwaite Water is an exceptionally well-monitored lake. Unfortunately, such detailed records of inflow and outflow nutrient loads are often unavailable at other sites and there may not be sufficient information available to calculate the sediment phosphorus flux by mass balance. The results of this investigation highlight the sensitivity of the phytoplankton community to internal load variation and the consequent need for it to be accurately represented in the model. It is therefore recommended that future versions of PROTECH are developed to include sediment phosphorus flux processes.

This study is the first use of PROTECH to investigate the impact of zooplankton grazing pressure on phytoplankton community structure and biomass. Phytoplankton grazing losses in PROTECH are calculated as a function of *Daphnia* energy requirements, growth efficiency, growth rate and feeding rate, along with the temperature sensitivities of each process (Reynolds *et al.* 2001). This formulation is an oversimplification of the processes that control zooplankton grazing pressure on phytoplankton in reality. According to the trophic cascade theory, planktivorous fish and macroinvertebrates exert a top-down control on zooplankton population size and species composition, which influences the grazing pressure exerted on the phytoplankton (Carpenter *et al.* 1985; Shapiro & Wright 1984). At present, PROTECH does not incorporate the loss of *Daphnia* due to consumption by fish or other predators into calculations of zooplankton grazing rate. In this study, the impact of planktivorous fish on phytoplankton community, via their predation of zooplankton, was tested by introducing a grazing rate multiplier.

However, the addition of a single trophic level to the model does not sufficiently represent the complexity of food web structure in Esthwaite Water, nor in many other lakes. In addition to healthy populations of planktivorous roach (*Rutilus rutilus*) and perch (*Perca fluviatilis*), the Esthwaite Water fish community comprises piscivorous pike (*Esox lucius*) and omnivorous farm-reared brown trout (Winfield *et al.* 2014). Simple linear food chain models predict that the regulation of primary producers by cascading trophic interactions is depended on food web structure, specifically the number of linkages (Hairston *et al.* 1960; Persson *et al.* 1988; Power 1992). It is hypothesised that the producer biomass in ecosystems with an odd number of trophic levels (i.e. phytoplankton, zooplankton and planktivorous fish) is resource limited as phytoplankton are released from grazing and permitted to grow as fast as nutrient availability and uptake permit. Conversely, in even-tiered systems (i.e. including piscivorous fish) zooplankton are free from predation and able to regulate phytoplankton biomass, regardless of nutrient availability. Although further work is needed to verify the predictions of food chain models, the limited data that is available from field studies (Carpenter *et al.* 2001; Hansson 1992; Persson *et al.* 1992), and enclosure experiments (Mazumder 1994) substantiates this theory. With this in mind, the current version of PROTECH is probably best suited to investigations based on lakes with a maximum of three trophic levels. The accuracy of phytoplankton community simulations could be improved by the inclusion of multi-level predation functions in the model.

The top-down effect exerted by a trophic level on the one below it does not solely depend on the vertical structure of the food web, but also on the horizontal structuring, i.e. heterogeneity within a trophic level (Leibold *et al.* 1997). Zooplankton community structure varies in relation to the seasonal succession of phytoplankton, changing abiotic conditions (e.g. temperature, trophic state), and predation pressure (George 2012; George *et al.* 1990; Yan *et al.* 2008). To gain a thorough understand of top-down phytoplankton regulation it is important to consider multiple zooplankton taxa. When PROTECH was first developed there was a scarcity of data pertaining to the grazing dynamics of most zooplankton groups (e.g. copepods, rotifers, ciliates). At the time only *Daphnia* data were sufficient for use in the formulation of grazing rate equations (Elliott *et al.* 2010). However, PROTECH is currently being developed to simulate zooplankton populations.

5.5 Conclusion

In summary, simulated phytoplankton communities responded to variations in both nutrient availability and zooplankton grazing rate. The relative importance of the two factors in regulating phytoplankton biomass varied seasonally in accordance with successional changes in plankton community structure, itself influenced by the interacting forces of zooplankton herbivory and nutrient limitation. However, the overall balance of control was dependent on the trophic state of the lake. When nutrient loads were high cyanobacteria dominated the phytoplankton community. Such conditions limited the potential for top-down regulation by herbivory due to food quality constraints on zooplankton. Thus, bottom-up control of total phytoplankton biomass by nutrient availability increased in importance. The proliferation of cyanobacteria due to eutrophication is detrimental to the ecological quality of a lake, and consequently threatens the provision of essential ecosystem services. The results of this study suggest that a decrease in cyanobacteria biomass can be achieved by reducing nutrient availability, whereas biomanipulation to increase zooplankton grazing pressure will have the opposite effect. Long-term simulations of phytoplankton community under conditions of increasing air temperature corroborate the theory that climate warming will exacerbate the effects of eutrophication. Nutrient loads to eutrophic lakes will need to be reduced substantially to mitigate against the impact of climate warming on phytoplankton communities. With regards to the management of Esthwaite Water, future strategies to reduce nutrient availability should focus on the internal phosphorus load, which was predicted to be of continued importance in sustaining high phytoplankton biomass following the recent reductions to external nutrient loads.

Chapter Six

General Discussion

6.1. Overview

Humankind relies on lakes for the provision of numerous essential goods and resources (Maltby & Ormerod 2011; Wetzel 2001). Unfortunately, lakes face a variety of pressures arising from catchment-based and in-lake anthropogenic activities and long-term changes in background environmental conditions, which greatly threaten their ecological integrity and the sustainability of important ecosystem services (Allan *et al.* 2013; Dudgeon *et al.* 2006; Vörösmarty *et al.* 2000). The response of lake ecosystems to external perturbations is controlled by the complex interaction of bottom-up and top-down forces (Maberly & Elliott 2012). A better understanding of how these processes interact, and of how their relative importance varies across different environmental conditions, is vital if they are to be effectively manipulated in the remediation of degraded lakes.

The main objectives of this study were:

1) to investigate the role of top-down and bottom-up processes, and their interactions, in regulating ecosystem structure and function; and
2) to determine how the relative importance of top-down and bottom-up processes changes in response to anthropogenic perturbations, specifically eutrophication and climate change.

Esthwaite Water, a eutrophic lake in the English Lake District, was selected for use as the model lake in this study. Water quality data have been collected from Esthwaite Water since the 1940s and human activities in the lake and surrounding catchment, such as sewage treatment processes and aquaculture, have been well documented (Maberly *et al.* 2011; Talling & Heaney 1983). Following decades of nutrient enrichment, several remediation measures were implemented in recent years in an attempt to attain EU Water Framework Directive (WFD) Good Ecological Status for Esthwaite Water. Essentially, lake managers unintentionally created an ecosystem-scale experiment in Esthwaite Water. Thus, presenting a relatively unique opportunity to disentangle the effects of different anthropogenic perturbations from one another and from the effects of wider environmental change.

It was hypothesised that the combined effects of nutrient enrichment and climate change would shift the balance of control towards bottom-up processes, and that the recovery of Esthwaite Water following remediation measures would be delayed by a combination of internal phosphorus loading and the exacerbation of eutrophic conditions due to climate change impacts.

Historical lake phosphorus budgets and potential drivers of water quality degradation were determined by analysing the long-term monitoring data in the context of changes to in-lake and catchment-based anthropogenic activities (Chapter Three). The current inventory of release-sensitive phosphorus in lake sediments was quantified, and the spatial variability of sediment phosphorus content and fractionation was assessed to determine the legacy of phosphorus inputs to the lake from ceased aquacultural operations (Chapter Four). Finally, the lake model PROTECH was used to gain a theoretical understanding of how Esthwaite Water, in its current ecological state, might react to potential remediation strategies and anthropogenic perturbations (Chapter Five).

The key finding of this study was that bottom-up rather than top-down forces were historically the main driver of water quality change in Esthwaite Water. Changes to the nutrient inputs, specifically phosphorus, appear to have caused the greatest amount of phytoplankton biomass variability. However, results suggest that community composition was influenced by both nutrient availability and zooplankton grazing pressure, the latter of which varied due to the cascading effect of trout stocking. As hypothesised, eutrophication and ongoing climate change shifted the balance of control further towards bottom-up processes. As a result, the recovery of Esthwaite Water following the implementation of remediation measures is predicted to be delayed by the combination of internal phosphorus loading and the exacerbation of eutrophic conditions due to climate change.

In this chapter, key findings with respect to the original research aims and hypothesis, and the wider body of research are discussed in further detail. Findings are used to inform lake management and remediation strategies, and suggestions for future research.

6.2. Synthesis of key findings

6.2.1. Top-down versus bottom-up control

Long-term environmental monitoring data collected from Esthwaite Water between 1970 and 2014 were analysed to determine the inter-annual drivers of water quality variability (Chapter Three). At the start of this period, Esthwaite Water, a naturally mesotrophic lake (May *et al.* 1997), already displayed symptoms of eutrophication (George *et al.* 1990; Talling & Heaney 1983). Increased overall phytoplankton abundance and the prevalence of cyanobacterial blooms during summer were attributed to a gradual increase in the external phosphorus load associated with the expansion of human activities in the

catchment since the mid-1800s (Bennion *et al.* 2000; Dong *et al.* 2011; McGowan *et al.* 2012). Unsurprisingly, data analysis revealed that from 1970 onwards phosphorus availability continued to be the main driver of total phytoplankton biomass, rather than grazing pressure exerted by cladoceran zooplankters or any of the climatic variables included in the analysis.

Simulations of phytoplankton communities conducted using the lake model PROTECH further confirmed the primacy of bottom-up rather than top-down control in Esthwaite Water (Chapter Five). Nutrient input and climate data from 2014 were used to drive the baseline simulation of phytoplankton community dynamics in Esthwaite Water over the course of one year. The combined effect of top-down and bottom-up pressures was tested by repeatedly running the baseline simulation through a step-wise combination of changes in nutrient load magnitude and *Daphnia* grazing rate. Simulated phytoplankton communities responded to variations in both nutrient availability and zooplankton grazing rate, but the response to changes in nutrient availability was considerably stronger. The relative importance of the two opposing forces in regulating phytoplankton biomass varied seasonally in accordance with successional changes in plankton community structure throughout the year, itself influenced by the interacting forces of zooplankton herbivory and nutrient limitation (George *et al.* 1990; George 2012; Sommer *et al.* 1986). However, the overall balance of control was dependent on the trophic state of the lake. When the overall nutrient load was high, cyanobacteria dominated the phytoplankton community. Such conditions limited the potential for top-down regulation by herbivory due to food quality constraints on zooplankton. Grazing on cyanobacteria is generally low due to their potential toxicity and low nutritional value (Ahlgren *et al.* 1990; Fulton & Paerl 1987; Lampert 1982), and the risk of filamentous or colonial morphologies clogging filtration appendages (DeMott *et al.* 2001; Gliwicz & Lampert 1990). Thus, bottom-up control of total phytoplankton biomass by nutrient availability increased in importance relative to top-down control by grazing.

Since 1970 the external phosphorus load to Esthwaite Water has fluctuated significantly. The installation of aquaculture cages for rainbow trout (*Oncorhynchus mykiss*) cultivation to Esthwaite Water in 1981 had a substantial and instantaneous impact on the lake phosphorus budget. The flux of uneaten fish food, excrement and sloughed scales from the cages to the surrounding water column effectively doubled the annual external phosphorus load to *c*. 2300 kg y^{-1} (Hall 1991; Holby & Hall 1991). The external phosphorus load to Esthwaite Water peaked at this point. Throughout the following decades various

measures were implemented to improve lake water quality and ecological condition. All of these schemes focussed on the bottom-up control of phytoplankton biomass by reducing external nutrient loads (Hall *et al.* 1993; Heaney & Butterwick 1989; Maberly *et al.* 2011). The most recent attempts at remediation involved the removal of aquaculture cages from the lake in 2009 and extensive upgrades to waste water handling and treatment facilities in the catchment between 2010 and 2012 (Maberly *et al.* 2011), which reduced the external load to its lowest value since 1970 (*c*. 560 kg y^{-1}). Total phytoplankton biomass (as indicated by chlorophyll *a*, μg l^{-1}) did not respond significantly to any of the major external phosphorus load changes between 1970 and 2014. Despite the overall reduction in external load, the desired improvements in water quality were not achieved.

6.2.2. The role of internal phosphorus loading

Annual net flux of phosphorus across the sediment-water interface was calculated as the difference of the input-output phosphorus budget (Chapter Three). It appears that as the external phosphorus load decreased, there was a gradual shift from annual net phosphorus uptake by lake sediments to net phosphorus release. This long-term pattern from 1970 to 2014 in sediment phosphorus flux is consistent with observations from many other shallow lakes where external phosphorus loading has been reduced (Jeppesen *et al.* 2005; Rossi & Premazzi 1991; Sas 1989; Søndergaard *et al.* 1999; Spears *et al.* 2012). The switch from net downward flux of sediment phosphorus to a net upward flux occurs because phosphorus sedimentation no longer outweighs the gross release of phosphorus from the sediment by a variety of biological and physico-chemical mechanisms (Søndergaard *et al.* 2001). In 2014 the internal phosphorus load accounted for approximately one third (*c*. 200 kg) of the total phosphorus load to Esthwaite Water, the majority of which was released during the main phytoplankton growing season (May to September). The apparent seasonal pattern of sediment phosphorus flux recorded by this study (Chapter Four) is typical of many lakes and has largely been related to phosphorus release mechanisms driven by temperature and biological activity (Jensen & Andersen 1992; Søndergaard *et al.* 1999, 2013; Spears *et al.* 2007b).

Liberation of phosphorus from lake sediments does not necessarily mean that it is instantly available for uptake by phytoplankton. When phosphorus is released from sediments into the hypolimnion it is relatively isolated from the mixed surface layer by the seasonal thermocline, and thus unavailable to a significant proportion of the phytoplankton biomass. Potential phosphorus supply mechanisms from the hypolimnion

to the epilimnion include diffusion across a static thermocline, boundary mixing from the breaking of internal waves and entrainment of hypolimnetic waters to the epilimnion during deepening of the mixed layer (MacIntyre *et al.* 1999; Soranno *et al.* 1997; Wodka *et al.* 1983). An investigation of phosphorus dynamics in Esthwaite Water by Mackay *et al.* (2014b) revealed that bioavailable soluble reactive phosphorus (SRP) transferred from the epilimnion to the hypolimnion by vertical diffusion and vertical entrainment during stratification. However, the relative importance of the two mechanisms varied throughout the season. Diffusive flux was more prevalent at the start of the summer, whereas entrainment was typically the dominant source throughout the late summer and autumn due to the gradual destratification and eventual overturn of the lake driven by wind mixing (Mackay *et al.* 2014b). Results presented in Chapter Three of this thesis would further suggest that sediment phosphorus released to the hypolimnion eventually becomes available to epilimnion-dwelling phytoplankton. A simple model was developed to investigate the importance of sediment-water phosphorus exchange in regulating phytoplankton biomass. The strong correlation between ΔChl (annual mean of measured chlorophyll *a* minus the annual mean of modelled chlorophyll *a*) and sediment phosphorus flux implies that the emergent internal phosphorus load was at least partly responsible for sustaining high phytoplankton biomass in surface waters following the recent reductions to external nutrient loads.

Following the reduction of external phosphorus load, a lag time of years to decades can elapse before water quality improvements are observed. Eventually, a new equilibrium state will be reached at which the net annual release of phosphorus from the sediment ceases, assuming no alteration to the external phosphorus loading during the transient period (Bailey-Watts & Kirika 1999; D'Arcy *et al.* 2006; Jeppesen *et al.* 1991, 2005; Søndergaard *et al.* 2007). The longevity and importance of the internal phosphorus load for overall lake phosphorus concentrations and overall water quality are mediated by lake characteristics such as hydraulic retention time (Spears *et al.* 2007b), pollution history (i.e. intensity and duration; Søndergaard *et al*. 2001), chemical characteristics of the sediment (Marsden 1989; Sas 1989) and biological activity at the sediment surface. For example, the magnitude of internal loading may diminish slowly as a result of phosphorus relinquishment from the lake by flushing or may be disrupted (capped) relatively quickly through the establishment of natural buffering systems at the sediment-water interface (e.g. macrophytes and benthic algae; Mehner *et al.* 2008; Scheffer *et al.* 2001).

On one hand, Esthwaite Water has a relatively short hydraulic retention time of around 83 days (Maberly *et al.* 2011), which could facilitate a speedy flush of legacy phosphorus from the lake (Dillon 1975). Conversely, the surface sediments of Esthwaite Water are extremely enriched with phosphorus relative to other UK lakes (Davies 1996; Spears *et al.* 2006) and up to 90 % is in a release-sensitive form. However, the maintenance of internal loading is ultimately dependent upon the external phosphorus supply. Providing that the external load does not increase again, the sediment phosphorus flux will eventually reach a new equilibrium at which point net phosphorus release will stop (Lijklema 1985; Marsden 1989; Sas 1989). During the period of aquacultural operations in Esthwaite Water, there was a localised increase in sediment phosphorus content around the site of the fish cages (Hall *et al.* 2001). The cages were removed from the lake in 2009. Just five years later the fish farm signal could no longer be detected in surface sediments collected from the site relative to sediment samples collected from an unimpacted site (Chapter Four). It is possible that the loss of this signal is an indication that phosphorus accumulated in lake sediments during the period of high external loading is now effectively being flushed from the system. Alternatively, the signal may have been masked by the uneven retention of phosphorus from another external source throughout the lake.

6.2.3. Impacts of climate change

Results of the analysis of long-term monitoring data from Esthwaite Water may provide insight into the potential impacts of climate change on lake ecosystem structure and function (Chapter Three). Annual mean air temperature increased significantly in the region around Esthwaite Water between 1970 and 2014. Although none of the water quality parameters included in the analysis displayed significant trends over the same period, there was a significant positive relationship between air temperature and relative cyanobacteria abundance. Further evidence for this relationship between temperature and cyanobacterial dominance comes from other long-term monitoring projects (Posch *et al.* 2012; Wagner & Adrian 2011; Weyhenmeyer 2001), comparisons between lakes in different climate zones (Kosten *et al.* 2012), laboratory and mesocosm experiments (De Senerpont Domis *et al.* 2007; Rasconi *et al.* 2017; Reynolds 2006), and studies of the impacts of heatwaves (Jöhnk *et al.* 2008). Modelling investigations, including the one conducted for this project (Chapter Five), have also established a link between warming and increased cyanobacteria abundance (Elliott 2010; Rolighed *et al.* 2016). The positive effect of temperature has partly been attributed to cyanobacteria having a higher thermal tolerance than eukaryotic phytoplankton (Butterwick *et al.* 2004). Additionally, the increased duration and strength of stratification with warming offers an advantage to

cyanobacteria (Wagner & Adrian 2011; Wilhelm & Adrian 2008). Some species have ecophysiological adaptations, such as buoyancy regulation (e.g. *Microcystis*) and the ability to fix atmospheric nitrogen (e.g. *Dolichospermum*), which give them a competitive advantage over other species in nutrient-depleted, poorly-mixed water column conditions (Carey *et al.* 2012; Paerl & Huisman 2008).

It is a widely held view that cyanobacteria are less susceptible to grazing by herbivorous zooplankton than other forms of algae (Ger *et al.* 2014; Visser *et al.* 2005). Consequently, the increased dominance of cyanobacteria due to climate warming could diminish top-down control on total phytoplankton biomass. The promotion of cyanobacterial blooms due to warming threatens to offset the positive impact of eutrophic lake remediation by nutrient load reduction (Wagner & Adrian 2009; Wilhelm & Adrian 2008) or biomanipulation (Jeppesen *et al.* 2010). PROTECH was used to test this theory by running multi-year phytoplankton community simulations under different scenarios of phosphorus load decrease and air temperature increase (Chapter Five). As hypothesised, air temperature increase did counteract the effect of declining phosphorus load. The response of total phytoplankton abundance and cyanobacteria abundance to reducing phosphorus availability was most pronounced when the air temperature did not increase over the course of the simulation. As the rate of warming increased, the response of phytoplankton to the declining phosphorus load diminished. In the scenarios that incorporated the largest air temperature increase over the modelling period (2 – 3 °C over 30 years), a point was eventually reached at which the effect of phosphorus load reduction was completely negated, and phytoplankton biomass began to increase with increasing air temperature.

PROTECH growth equations are formulated to account for the direct effect of temperature variability on phytoplankton abundance and community structure, as well as the indirect effects via changes in lake thermal structure and *Daphnia* grazing rate (Reynolds *et al.* 2001). However, climate change is likely to impact on multiple components and processes of lake ecosystems that have not been considered in this study but have the potential to drive water quality degradation. For example, climate warming is expected to trigger changes in fish community structure through direct and indirect effects on fish metabolism, biotic interactions and thermal tolerances affecting geographical distribution. All of these changes are predicted to increase predation pressure on zooplankton and macroinvertebrates, which will resemble, and may intensify, the effects triggered by eutrophication (Jeppesen *et al.* 2007, 2010). Higher internal phosphorus

loads may also reinforce eutrophication due to an increase in temperature-mediated phosphorus release from sediments (Jensen & Andersen 1992; Jeppesen *et al.* 2009; Kosten *et al.* 2011).

6.3. Lake management implications

Although eutrophication is the primary cause of ecological degradation in many lakes, it is clear from the findings of this study and numerous others, that simply removing or reducing external phosphorus loads does not often result in immediate water quality improvement (D'Arcy *et al.* 2006; Hall *et al.* 1993; Jeppesen *et al.* 1991; Søndergaard *et al.* 2007; Welch & Cooke 1995). In such cases, it is possible that external load reductions were not sufficient to lower the in-lake phosphorus concentration below a critical threshold value at which phytoplankton growth would be limited. Fastner *et al*. (2016) reviewed the case histories of eight lakes in Europe and the United States, which had been subjected to long-term reductions in phosphorus inputs. In all cases, algal blooms did not respond until total phosphorus decreased below some threshold value, usually around 50 $\mu g\ l^{-1}$, at which point nutrients rather than light limited phytoplankton production (Reynolds 2002; Sas 1989). Where previous efforts appear to have failed, further reductions to the external phosphorus load may be all that is required to reach this threshold and instigate an improvement in ecological conditions (Schindler *et al.* 2016). However, the response of eutrophic lakes to remediation measures is usually more complex (Spears *et al.* 2017). Internal feedback mechanisms established under conditions of high external loading may increase the resilience of lake ecosystems to changes in external pressures (Ibelings *et al.* 2007; Scheffer *et al.* 1993, 2001).

The theory of alternative stable states predicts that ecosystems have more than one equilibrium (Lewontin 1969; May 1977; Scheffer *et al.* 2001). Many observations support the existence of two alternative stable states or equilibria in shallow lakes (Blindow *et al.* 1993; Dent *et al.* 2002; Dokulil & Teubner 2003; Korner 2001; Moss *et al.* 1996). At low nutrient levels only macrophyte dominated clear water conditions can exist, whereas a phytoplankton dominated turbid water equilibrium prevails when nutrient levels are high (Scheffer *et al.* 1993). A number of feedback mechanisms exist that buffer the respective states against change following a disturbance, e.g. an alteration to the external phosphorus load. For instance, in the phytoplankton-dominated turbid state, fish populations tend to be dominated by benthivores and zooplanktivores. Zooplanktivores diminish top-down control of phytoplankton by exerting high grazing pressure on zooplankton (Jeppesen *et al.* 1997) and the feeding habits of benthivorous fish facilitate

the resuspension of sediment to maintain the turbid state. In this situation, light limitation and disturbance of the sediment make it difficult for submerged plants to settle (Scheffer *et al.* 2003). Another factor that stabilises the turbid state is the release of phosphorus from the sediment, which fuels phytoplankton growth until sediment phosphorus flux re-equilibrates with the reduced external load (Søndergaard *et al.* 2003). Conversely, macrophytes are central to the stabilisation of the clear water state following an increase in phosphorus loading. They reduce wind-induced sediment resuspension, often associated with sediment phosphorus release (Madsen *et al.* 2001), and provide a refuge for zooplankton against predation by fish, which enhances the top-down control of phytoplankton by zooplankton grazing (Jeppesen *et al.* 1998; Scheffer *et al.* 1997; Schriver *et al.* 1995).

On account of these stabilising factors, lakes are most resilient to perturbations either at very low nutrient concentrations or very high nutrient concentrations. Resilience is weakened at intermediate nutrient concentrations following nutrient load reductions, making the ecosystem vulnerable to a state shift (Lowe *et al.* 2001; Scheffer *et al.* 2001). This theory of alternative stable states offers an explanation for the persistence of poor water quality in many lakes despite considerable reductions to external nutrient loads. Following unsuccessful attempts to improve water quality by external phosphorus load reduction, managers of turbid lakes could capitalise on the potentially unstable conditions by implementing measures to disrupt the stabilising feedback mechanisms and force a shift in state to the macrophyte dominated clear water equilibrium (Scheffer *et al.* 1993).

However, it is also possible that not all lakes exhibit regime shifts and that reorganisation at the ecosystem scale is either linear or follows a path of gradual adjustment as the pressures change (Spears *et al.* 2017). The current argument against regime shifts and alternative stables states claims that the theory is not yet fully developed due to a lack of convincing empirical evidence and appropriate data to underpin it. Evidence is mainly limited to shallow temperate lakes (Capon *et al.* 2015). Genkai-Kato & Carpenter (2005) concluded that the likelihood of obtaining a regime shift from turbid to clear water state is lowest in lakes of intermediate depth, and restoration of such lakes is therefore difficult. Their reasoning was that in shallow lakes there is good potential for the occurrence of high areal macrophyte cover, which will help maintain the clear water state, and that in deep lakes the stratification and separation of the water may limit the impacts of internal phosphorus loading on the photic zone. Although Esthwaite Water is classified as a 'shallow lake' under the EU WFD, with a mean depth of 6.9 m and maximum depth of 16

m, it would likely qualify as an intermediate depth lake in comparison to many of lakes in which regime shifts have apparently been observed.

Remediation efforts may be hampered due to the impacts of other interacting processes, climate change for example. The results of this study contribute to a growing body of evidence that climate change exacerbates the effects of eutrophication by various mechanisms (Jeppesen *et al.* 2010; Moss *et al.* 2011; Paerl & Huisman 2008). It seems likely that increasingly favourable growing conditions may be responsible for the continued dominance of cyanobacteria in Esthwaite Water, and indirectly for the maintenance of high overall phytoplankton biomass, despite external phosphorus load decline over the last two decades. Greater improvements in water quality may have occurred had climate warming not been concurrent with phosphorus load reductions. In the face of ongoing and accelerating climate change (IPCC 2014a), nutrient control will have to be intensified just to prevent further degradation, let alone make improvements to water quality (Moss *et al.* 2011; Trolle *et al.* 2011).

The EU WFD focuses on traditional stressors of waterbodies, such as eutrophication, organic pollution, and acidification (European Union 2000). As yet, the effects of climate change and other emergent stressors (e.g. invasive species, new toxic substances and siltation) have not formally been factored into the mitigation strategies for pre-existing environmental degradation (Hering *et al.* 2010). There is growing concern that the mutual effects of climate change and nutrient enrichment will mean that the existing reference conditions used as targets of recovery will become more difficult to attain and increasingly invalid (Bennion *et al.* 2011; Moss *et al.* 2011).

6.4. Potential lake remediation measures

In recent decades limnological research has largely focussed on the investigation and development of measures to mitigate against and counteract the impact of human-induced stressors on lake ecosystems (Harper 1992; Jeppesen *et al.* 2012; Mackay *et al.* 2014a; Søndergaard *et al.* 2007, 2008; Zamparas & Zacharias 2014). A multitude of potential strategies now exist for the remediation of eutrophic lakes such as Esthwaite Water, a selection of which are discussed below.

6.4.1. Bottom-up control: Phosphorus load reduction

The majority of remediation projects have initially focussed on reducing eutrophication by controlling external nutrient loads, with a particular focus on phosphorus as it is the

main nutrient limiting phytoplankton growth in many lakes (Schindler 1977; Vollenweider 1968). The major sources of phosphorus to lakes are sewage and industrial effluents (point sources), and agricultural run-off (diffuse sources). The identification and mitigation of point sources is relatively easy and in recent years they have become decreasingly important in Europe due to more sophisticated waste water treatment techniques, while diffuse sources are a continuing and unresolved problem (Kristensen & Hansen 1994). This pattern is reflected to some degree in the long-term nutrient budgets for Esthwaite Water (Chapter Three). With the upgrades to Hawkshead Wastewater Treatment Works (WwTW) and the removal of the fish cages from the lake, the scope for additional reductions of point source loads is limited at this time.

The diffuse phosphorus load from the catchment has also been in decline since peak loading in the 1970s, probably as a result of the DEFRA-funded Catchment Sensitive Farming initiative and other environmental stewardship schemes adopted by local land owners in recent years. Techniques include, soil conservation, land use management and careful nutrient and pesticide planning (Collins *et al.* 2007; Sharpley *et al.* 1994). Further reductions to the catchment diffuse load could also be achieved by the establishment of riparian and other buffer zones around the lake (Mitsch *et al.* 1995; Williamson *et al.* 1996). However, PROTECH analysis of the sensitivity of total phytoplankton biomass to variability in the individual phosphorus loads, suggests that reducing any one of the three main external loads would have a negligible effect on annual mean chlorophyll *a* concentration (Figure 6.1). Although catchment load accounts for the largest portion of the total annual load (*c*. 60 %), and could reasonably be expected to affect water quality, its delivery to the lake is associated with rainfall and is therefore relatively low in the summer during the main phytoplankton growing season (Heathwaite & Dils 2000). Efforts to further reduce phosphorus loading from these sources may be futile in the promotion of water quality improvements.

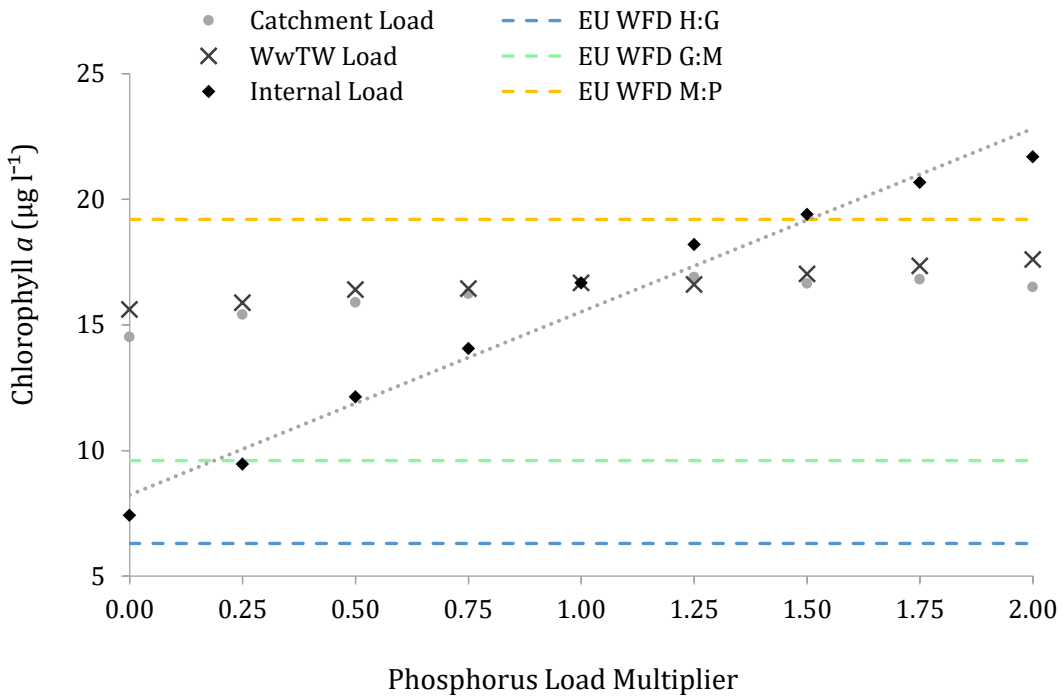

Figure 6.1: Sensitivity analysis of PROTECH simulated phytoplankton chlorophyll *a* to individual phosphorus loads. Coloured lines show the EU WFD High (H), Good (G), Moderate (M) and Poor (P) status boundaries specific to Esthwaite Water for annual mean chlorophyll *a*.

On the other hand, manipulation of the internal load used to drive PROTECH simulations produced substantial variation in the annual mean chlorophyll *a* concentration. A reduction to 25 % of the current (2014) load would be sufficient to achieve EU WFD Good Ecological Status for chlorophyll *a* (Figure 6.1). In contrast to the external load, the internal phosphorus load has a large impact relative to its size because release of phosphorus from the sediments to water column predominantly occurs during the phytoplankton growing season. Internal phosphorus loading will gradually decrease as Esthwaite Water approaches a state of equilibrium with the reduced external load. However, this transient period could last decades (Sas 1989; Søndergaard *et al.* 1999, 2013; Spears *et al.* 2007a). Consequently, it is recommended that future management strategies for Esthwaite Water are focused on controlling the internal phosphorus load to accelerate ecological recovery.

A variety of techniques exist for reducing internal phosphorus load. The removal of phosphorus-rich sediment from Esthwaite Water by dredging has previously been considered to reduce internal phosphorus release but it was deemed to be too expensive and logistically difficult (Hall *et al.* 2000). Other potential techniques include: artificial

mixing or hypolimnetic oxygenation to improve phosphorus binding potential (Beutel & Horne 1999; Gächter & Wehrli 1998); the application of phosphorus-capping agents (e.g. Phoslock® or aluminium salts) to form a stable mineral that is subsequently retained in the sediment (Gibbs *et al.* 2011; Meis *et al.* 2013; Spears *et al.* 2013b); or hypolimnetic withdrawal, the selective discharge of bottom water to enhance the removal of nutrients that build up when the hypolimnion becomes anoxic (Nürnberg 2007).

Unfortunately, none of these methods to accelerate lake recovery are entirely reliable or without problems. Apart from the logistical and financial issues associated with dredging, it has been found to have poor long-term effectiveness. Although phosphorus-rich sediments are removed from the system, the newly exposed sediment may have a low phosphorus-sorption capacity, leading to an accumulation of phosphorus in the water column (Søndergaard *et al.* 2007). Similarly, the application of Phoslock®, a lanthanum-bentonite modified clay, and other phosphorus-capping agents have also been shown to have an immediate positive impact on water quality but low long-term sustainability (Boers *et al.* 1992; Reitzel *et al.* 2005). In theory, the lanthanum in Phoslock® will react with phosphate in the water column to form the stable mineral rhabdophane, which effectively immobilises it in bed sediments. However, the retentive capacity of Phoslock® varies across different physico-chemical conditions. For example, an increased dose of Phoslock® would be required under anaerobic conditions compared to aerobic conditions (Meis et al. 2013). Additionally, a lack of environmental quality standards and peer reviewed ecotoxicological studies currently hamper the assessment of the ecological impact of lanthanum leaching (Spears *et al.* 2013b).

As for hypolimnetic oxygenation, it can reduce the accumulation of phosphate in deep waters, but it needs to be continuous to avoid the return of anoxic conditions, reduction of iron oxides and the consequent release of phosphorus to the water column (Søndergaard *et al.* 2007). Additionally, as Gächter & Müller (2003) highlighted, the retention of phosphorus in sediments is not solely dependent on redox conditions at the sediment surface. Permanent phosphorus burial only occurs if, because of depressed sulphide production, the molar ratio of ferrous phosphate to iron sulphide is high in the anoxic sediment layers. High demand for electron acceptors in the anoxic sediment could lead to sulphate reduction and the release of sulphide. Sulphide can dissolve ferrous phosphates and precipitate the Fe(II) as iron sulphide. Hence, iron sulphide formation could increase the phosphate concentration and prevent association of iron with phosphate in the anoxic sediment pore water (Gächter & Müller 2003). Therefore, the

success of hypolimnetic oxygenation is linked to the nature and supply of organic matter, particulate iron and sulphate in sediments.

Given that the net release of phosphorus from lake sediments will gradually decline following the control of external loads (Sas 1989), it is for lake managers to decide if attempting to engineer a faster recovery outweighs the risks, cost and effort associated with any of the strategies mentioned above. Or, if the law of diminishing returns dictates that it would be more sustainable to simply wait-out the re-equilibration phase.

6.4.2. Top-down control: Manipulation of trophic dynamics

Biomanipulation has often been employed to combat the effects of eutrophication, either alongside or instead of the more traditional bottom-up approach of reducing external nutrient load (Potthoff *et al.* 2008; Skov & Nilsson 2007; Søndergaard *et al.* 1990, 1997). It is based on the fundamental assumption that a change in predator biomass at the highest trophic levels of an aquatic food web cascades down to the lowest level (Carpenter & Kitchell 1993; Shapiro *et al.* 1982). In theory, if planktivorous fish become scarcer, zooplankton grazers will increase in abundance and phytoplankton biomass will decline. The implementation of top-down measures is relatively straight forward. Either, planktivorous fish, e.g. roach (*Rutilus rutilus*) and/or perch (*Perca fluviatilis*), are selectively removed, or piscivorous fish, e.g. pike (*Esox lucius*), are introduced to increase predation pressure on planktivores (Jeppesen *et al.* 2012).

Marked improvements in water clarity and phytoplankton biomass have been recorded in many lakes immediately following food web manipulation (Potthoff *et al.* 2008; Søndergaard *et al.* 2008). However, biomanipulation projects are frequently observed as having poor long-term effects. Lake morphology, trophic status and food web structure all factor in the success of biomanipulation. Whole-lake experiments have demonstrated that in lakes dominated by planktivorous fish, phytoplankton biomass increases in response to pulses of nutrient input from external sources. On the other hand, when a lake is dominated by piscivorous fish, and therefore has a longer food chain, nutrient pulses are more effectively transferred to higher trophic levels because zooplankton are able to control phytoplankton biomass under conditions of relatively low grazing pressure (Carpenter *et al.* 1992, 1996; Schindler *et al.* 1996). Additionally, in lakes with a longer food chain the phosphorus return rate is slower because large fish act as longer-term phosphorus sinks (Carpenter & Cottingham 1997). Despite being stocked with trout, Esthwaite Water is currently dominated by planktivorous roach and perch (Winfield *et al.*

2014), therefore manipulating the fish community to increase the ratio of piscivores to planktivores could instigate water quality improvements.

Top-down control is more effective in shallow lakes than it is in deep lakes because macrophytes are able to colonise relatively large areas and the potential for a shift towards clear water equilibrium is consequently greater (Benndorf *et al.* 2002; Jeppesen *et al.* 1997). As demonstrated by the PROTECH simulations presented in Chapter Five, the response of total phytoplankton abundance to top-down control is enhanced under conditions of low nutrient availability. The likelihood of forcing a state shift to clear water and macrophyte dominance by biomanipulation is greatest in shallow lakes where nutrient availability has already been subject to control. Although Esthwaite Water may be more susceptible to a state shift following recent phosphorus load reductions, it is probably not the ideal candidate for remediation by top-down control. Pearsall's survey of Esthwaite Water in the 1910s recorded a macrophyte depth limit of around 2 m (Pearsall 1917), equivalent to approximately 20 % of the surface area of the lake (Mackay *et al.* 2012). The inability of macrophytes to colonise a larger area at this site may impede stabilisation of the mechanisms necessary to maintain a clear water equilibrium. Additionally, the high abundance of cyanobacteria relative to other phytoplankton in Esthwaite Water may inhibit effective top-down control on total phytoplankton biomass for the reasons previously discussed.

6.4.3. Minimising climate change impacts

Ideally, the impacts of climate change on lakes and other ecosystems would be mitigated by a world-wide commitment to reducing net emissions of carbon dioxide, methane and other greenhouse gases to the atmosphere (IPCC 2014c). However, environmental protection is a low priority on global and national political agendas. The implementation of measures to reduce emissions is often side-lined in favour of securing short-term economic growth, food security and job security, as exemplified by the United States' intention to withdraw from the Paris Climate Accord under pretences of job creation in the coal sector (Crooks 2017). Switching the focus of climate change mitigation from global to local stewardship could be an option (Tang *et al.* 2010). For example, community-led organisations in the Lake District have facilitated land use changes to increase carbon sequestration (Windermere Reflections) and funded the reintroduction of hydropower to the area (Lake District Sustainable Development Fund). Unfortunately, poor government investment and a reluctance of community members to participate often hinders their success. Increased public awareness of the threat that ongoing climate

change poses to the sustainability of essential ecosystem services is required to incentivise communities and policy makers to take mitigatory action.

Even if existing greenhouse gas concentrations could be immediately stabilised, global mean air temperature would continue to increase by an estimated 0.6 °C this century, relative to 1980 to 1999. This is because of the long timescales over which some climate feedback mechanisms operate (Wuebbles *et al.* 2017). With this in mind, the emphasis for lake managers should be on adaptation to climate change, as well as mitigation of the causes. Given that one of the major consequence of climate change on lake ecosystems is an exacerbation of eutrophic conditions, any of the remediation measures described in the above sections could be applied (Jeppesen *et al.* 2009; Moss *et al.* 2011; Rolighed *et al.* 2016).

6.5. Importance of ecosystem-scale experiments

There are four main approaches that scientists use to investigate ecosystems: theoretical (e.g. using conceptual, computational or mathematical models), comparisons of contrasting ecosystems, observations of natural fluctuations during long-term studies, and experiments that deliberately manipulate specific variables under controlled environmental conditions (Holling 1995; Likens 1985, 1989; Pickett *et al.* 2007). These four approaches have complementary strengths and weaknesses. Therefore, most investigations employ a combination of two or more of these methods (Carpenter 1998), as was the case in this study. One of the main advantages of conducting experiments is that they allow for a more causal understanding. Whereas theoretical, comparative and long-term studies provide context for the development of hypotheses, only experimentation can definitively eliminate potential explanations for ecosystem change.

Most ecological experiments involve the isolation and manipulation of a small part of an ecosystem, for example in a microcosm or mesocosm. However, important processes and features of whole ecosystems are often missing from small-scale experiments, which may limit the range of possible responses (Carpenter 1998; Schindler *et al.* 2008). For example, components of aquatic ecosystems that may be difficult to incorporate into microcosm and mesocosm experiments include the activities of wide-ranging organisms (e.g. migratory fish), air-water and sediment-water exchanges, cross-habitat interactions (e.g. littoral-pelagic, benthic-pelagic), and the natural variability of physiological conditions (e.g. thermal structure, light attenuation, mixing). Conversely, ecosystem-scale experiments are conducted *in situ* and are designed to cover such a spatial and temporal

scale as to include most, if not all, processes of an ecosystem (Carpenter 1998; Fayle *et al.* 2015; Schindler 1998). Ecosystem-scale experiments have been carried-out on a wide range of ecosystems, including forests (Beier & Rasmussen 1994; Emmett *et al.* 1998), oceans (Coale *et al.* 1996), wetlands (Mitsch *et al.* 1995, 1998; Woodwell *et al.* 1979) and lakes (Carpenter *et al.* 2001; Harris *et al.* 2007; Schindler 1974). The results of these investigations are closer to realism than those obtained from simplified small-scale experiments and are therefore directly applicable to environmental policy and management.

Ecosystem-scale experiments are responsible for some of the most significant advances in our understanding of the structure and functioning of lake ecosystems. The Experimental Lakes Area (ELA) is a research station in Ontario Canada, which was founded in 1968 and encompasses 58 formerly pristine freshwater lakes (Brunskill & Schindler 1971). It has hosted many of the most influential whole-lake experiments (Schindler 2009b). The most significant perhaps was the nutrient fertilisation experiment of Schindler (1974) in which one half of a partitioned lake was dosed with carbon and nitrogen, and the other half with phosphorus in addition to carbon and nitrogen. The development of highly eutrophic conditions in the basin with added phosphorus but not in the part of the lake receiving only carbon and nitrogen provided strong evidence for phosphorus as the limiting nutrient in lake ecosystems. Previously, many scientists believed that carbon controlled eutrophication in lakes, a view that was founded largely on the results of bottle bioassay experiments where phytoplankton growth was stimulated with the addition of carbon but not nitrogen or phosphorus (Kerr *et al.* 1970; Kuenzel 1970; Lange 1970). However, it was apparent from the whole-lake experiment that bottle bioassay experiments provided misleading evidence by excluding the process of atmospheric carbon dioxide invasion. In natural waterbodies, this process typically provides sufficient carbon to support phytoplankton populations proportional to phosphorus availability (Schindler 1971; Schindler *et al.* 1972). These findings defined the trajectory of limnological research and lake management in the following decades (Harper 1992; Persson *et al.* 1988; Sas 1989). Additionally, the now iconic aerial photographs, which captured the bright green algal blooms of the phosphorus-treated side of the lake in stark contrast to the clear water of the untreated side, convinced policy makers and the public that phosphorus inputs to freshwater systems needed to be controlled (Schindler 2009b).

Despite the demonstrable advantages of whole-lake experiments, their use appears to be declining. They may be losing favour because they are often difficult to execute and not easily replicated. Many ecologists favour small-scale experiments that are easily replicated to produce data suitable for rigorous statistical analysis, even though they do not accurately represent the structure and function of natural ecosystems (Schindler 1998). There are also very few lakes in developed countries that are free from conflicting interests and available to use exclusively for research purposes. However, given the current climate of financial uncertainty and austerity, a lack of funding for environmental research is probably the most significant obstacle to conducting ecosystem-scale experiments, as exemplified by the termination of federal funding to the ELA in 2013 (Hoag 2012).

Increasingly, ecosystem-scale experiments are being conducted unintentionally. Many studies document the impact of inadvertent disturbances on ecosystems, caused by either human activity or natural events (Diamond 1983; Hille Ris Lambers *et al.* 2013; Rogers *et al.* 2012; Sagarin & Pauchard 2010). When pre-disturbance data exists, against which to compare post-disturbance data, the study site can effectively be utilised as an ecosystem-scale experiment. Unintentional ecosystem-scale experiments are especially useful if the site is monitored regularly as part of an ongoing long-term programme. In this scenario, natural ecosystem variability and the effects of global or regional-scale environmental change (e.g. climate warming) can be disentangled from the effects of the unintentional disturbance (Likens 1989; Mills *et al.* 2017; Willis & Birks 2006). Notable examples of unintentional ecosystem-scale experiments include the effect of wolf extirpation on food-web dynamics in Yellowstone National Park USA (Beschta & Ripple 2006) and the impact of overfishing on Jamaican coral reefs (Hughes 1994).

The investigation of human-induced disturbances to Esthwaite Water presented in this thesis is another example of an unintentional ecosystem-scale experiment. Although operational changes at Hawkshead WwTW and Esthwaite Water Trout Fishery were not intended for research purposes they can be considered as experimental treatments because ecological monitoring data exist for the periods before and after the disturbances. One of the main aims of this thesis was to investigate the impact of trout stocking on lake structure and function. It would have been almost impossible to determine accurately the effects of trout stocking using a smaller-scale experimental set-up due to their relatively large size and high mobility. Additionally, small-scale experiments and ecosystem models typically assume that when a natural system is perturbed it will respond in a predictable

manner based on a theoretical understanding of the relevant ecological processes. Consequently, they represent a simplified version of the ecosystem in question. For example, PROTECH assumes that grazing pressure on phytoplankton is exerted exclusively by the cladoceran zooplankton *Daphnia*. However, analysis of the long-term monitoring data for Esthwaite Water revealed unexpected trophic interactions (Chapter Three). Prior to analysis it was expected that the effect of trout stocking on phytoplankton would be due to changes in the predation pressure on zooplankton, which would in turn alter zooplankton grazing pressure on the phytoplankton. While the results show that trout stocking did have a significant impact on phytoplankton biomass and community structure, the trophic cascade triggered by stocking appears to have bypassed zooplankton via another herbivorous component of the lake food web. It is unlikely that such food web complexity would have been revealed by alternative ecosystem investigation methods. These findings highlight the importance of ecosystem-scale experiments, be they intentional or unintentional. Given the apparent decline in the implementation of intentional manipulative ecosystem-scale experiments, it is vital that opportunities to investigate the impacts of unintentional disturbances are exploited to continue advancing our understanding of ecosystems.

6.6. Suggestions for future work

The research presented in this thesis contributes to our understanding of the impact of multiple human-induced stressors on the structure and function of lake ecosystems and the effectiveness of consequent remediation measures. However, further research is required if ecological quality and associated ecosystem services are to be secured in Esthwaite Water and other similarly impacted lakes. The following suggestions for future work could advance topic knowledge and improve the accuracy of the results and conclusions presented here.

Numerous factors besides those discussed in this thesis have the potential to drive water quality change, for example: pH (Schindler *et al.* 1985; Steinberg & Wright 1994), toxic substances (Olszewska *et al.* 2016), organic pollution (Williamson *et al.* 2016), invasive species (Pejchar & Mooney 2009; Strayer 2010) and the availability of nutrients other than phosphorus (Elser *et al.* 2007). Broadening the range of parameters routinely monitored in Esthwaite Water, and lakes in general, could expand on our current understanding of ecosystem functioning. In terms of nutrients, phosphorus is often assumed to be the main nutrient limiting phytoplankton growth and is used to drive eutrophic lake restoration programmes (Marsden 1989; Schindler *et al.* 2016). This

assumption is validated by the meta-scale relationship between phosphorus and chlorophyll *a* (Vollenweider 1968). However, there is considerable evidence that nitrogen is limiting on occasion or at certain sites (Elser *et al.* 2007; Maberly *et al.* 2002; May *et al.* 2010). Burson *et al.* (2016) reported a shift from nitrogen to phosphorus limitation in a marine setting resulting from an unbalanced reduction in nutrient loads. Whether current efforts to tackle eutrophication in freshwaters by controlling phosphorus inputs could increase the nitrogen to phosphorus ratio to such an that it causes a shift towards nitrogen limitation may be an interesting line for future research. Therefore, both elements should be considered in future lake and catchment management plans as there may be situations where a reduction of the nitrogen load could serve as a supplementary tool to achieve water quality improvements.

Trophic status and climate conditions are both known to be key controls of fish community composition and structure (Jeppesen *et al.* 1998, 2010; Olin *et al.* 2002). Unfortunately, it was not possible to investigate the effects of environmental change on the fish population of Esthwaite Water as fish surveys are not routinely conducted at the lake as part of the long-term monitoring programme. Future research could focus on the generation of historical fish population data, which could be used in conjunction with long-term monitoring data to gain a better understanding of the importance of top-down processes in water quality control. The use of traditional palaeolimnological techniques involving the assessment of sub-fossil cladocera remains in lake sediments may be used to trace changes in fish predation, which would provide a proxy for zooplanktivorous fish abundance (Jeppesen *et al.* 2002; Korosi *et al.* 2013). Alternatively, the analysis of ancient DNA extracted from lake sediments may allow retrospective molecular monitoring of fish populations. Fish DNA extracted from lake water (Hänfling *et al.* 2016) has been successfully validated against catch data for contemporary fish populations but refinement of the methodology is required before it can be applied to down-core sediment samples.

The results of the long-term data analysis (Chapter Three) suggest that cyanobacteria abundance increased significantly following the start of trout stocking to the lake for game fishing. However, the trophic interactions that lead to this increase are not obvious. A combination of stable isotope analysis and trout gut contents analysis could be used to establish current food web dynamics in Esthwaite Water (Grey *et al.* 2002; Jones & Waldron 2003). This could help to inform future decisions on lake management. For

example, whether the discontinuation of farmed trout stocking will lead to water quality improvements.

In Chapter Three the impacts of long-term change in average climate conditions on water quality were investigated. In addition to warming and increased overall rainfall, it is predicted that the intensity and frequency of storm events and periods of drought will increase in the UK with global climate change (George *et al.* 2007; Murphy *et al.* 2009). Short-lived extremes of rainfall, temperature and wind speed can have a significant effect on lake ecosystems. For example, high rainfall events may initially prevent algal bloom-formation because of enhanced flushing. But elevated loads of nutrients and turbidity from terrestrial sources could promote the growth of shade-adapted cyanobacteria once high discharge subsides (Paerl & Otten 2013). The impacts of an increase in such events are likely to differ from the effects of long-term gradual climate change (Mantzouki *et al.* 2016). Future work could focus on gaining a better understanding of the effects of these extreme events on lake physiology and the consequences for phytoplankton and the wider lake community.

Finally, the work presented in this thesis would not have been possible without access to the long-term monitoring data for Esthwaite Water. Hopefully this thesis has demonstrated the importance of such records in limnological research. They often provide a unique opportunity to investigate the impacts of human activities on the structure and functioning of lake ecosystems, and consequently to implement appropriate management measures to protect the essential goods and resources that we depend upon. It is imperative that lake monitoring programmes are secured into the future. If so, this study can be revisited following the EU WFD deadline to assess whether climate change and internal phosphorus loading do indeed prevent the recovery of eutrophic lakes to Good Ecological Status following external nutrient load reductions.

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Catchment Area

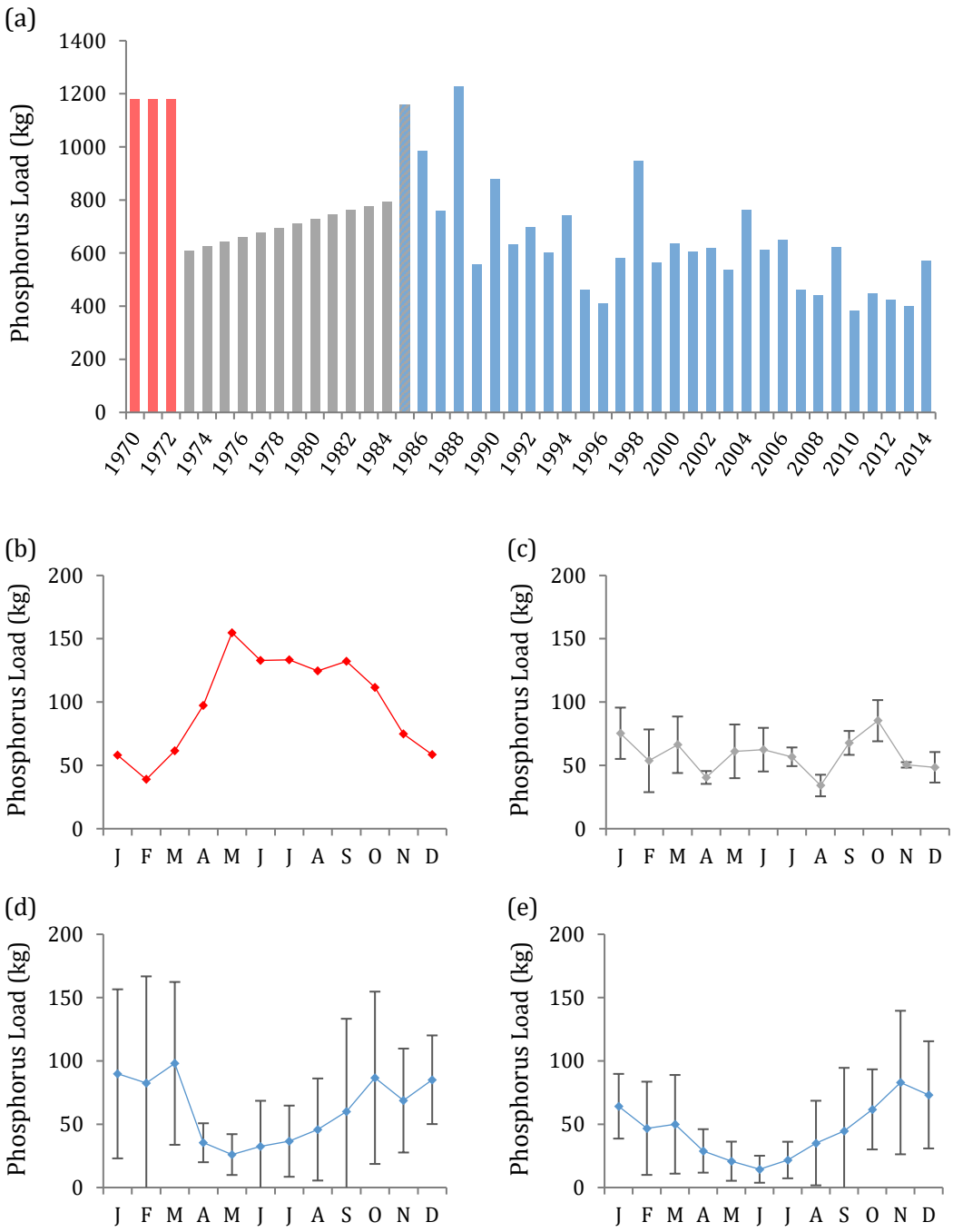

Figure A.1. (a) Annual catchment phosphorus loads from 1970 to 2014 and average monthly loads between (b) 1970 and 1972, (c) 1973 and 1984, (d) 1985 and 1999, and (e) 2000 and 2014. Error bars represent standard deviation of the mean.

1970 – 1972: F.J.H. Mackereth calculated monthly soluble reactive phosphorus (SRP) loads (kg) from Black Beck and the five minor inflows to Esthwaite Water from June 1968 to June 1969 (Talling & Heaney 1983). It was considered unlikely that the catchment phosphorus load would have changed significantly between 1968 and 1972 as there is no record of alterations to catchment-based activates or processes that could have impacted on the phosphorus load (e.g. livestock or agricultural practices, sewage treatment, population size). Therefore, the 1968-69 monthly estimates of load were applied to 1970 to 1972. Black Beck SRP and total phosphorus (TP) concentrations measured since the start of long-term monitoring in 1985 are strongly correlated (r = 0.75, p < 0.001). This linear relationship was used to convert the SRP loads estimated by Mackereth to TP loads (Figure A.1b).

1973 – 1984: Hawkshead WwTW was established in 1973 to improve sewage treatment facilities in the area. Prior to this, many households within the Esthwaite Water catchment area would have been served by septic tanks, which are often a significant point source of phosphorus to nearby waterbodies (May *et al.* 2015). It is likely that the centralisation of sewage treatment facilities resulted in a decrease in the catchment phosphorus load. This decrease was accounted for by subtracting monthly estimates of WwTW phosphorus load from the corresponding monthly catchment loads for 1972. Catchment area phosphorus loads from January 1974 to March 1985 were then obtained by linear interpolation between the 1973 monthly estimates and average monthly loads calculated from 1986 to 1990 load estimates (Figure A.1c).

1985 – 2014: TP concentration data for Black Beck was available from the long-term monitoring programme from April 1985 onwards. Monthly mean values of Black Beck TP concentration (TP_{BB}) were calculated for use in Equation 3.1. Black Beck discharge is not measured routinely as part of the long-term monitoring programme. However, a strong correlative relationship was established between measurements of Black Beck discharge and outflow (Cunsey Beck) discharge taken throughout 2013 and 2014 (r = 0.75, p < 0.001). This relationship was used to derive Black Beck discharge (Q_{BB}) from the NRFA long-term record of Cunsey Beck discharge for use in phosphorus load calculations.

Apart from monthly measurements of TP concentration and discharge collected throughout 2013 and 2014, the five minor inflows to Esthwaite Water have been subjected to relatively little monitoring. Initially, it was intended that the correlative

relationship between monthly Black Beck and minor inflow phosphorus loads throughout 2013 and 2014 would be used to backfill the minor inflow load record for the rest of the study period. However, no reliable relationship existed between these measures (r = 0.48, p = 0.19). Instead, the minor inflow phosphorus load was assumed to be 14 % of inflow load based on the findings of two previous studies (Hall *et al.* 1993; Talling & Heaney 1983) and total catchment load was calculated accordingly (Equation 3.2, Figure A.1d/e).

Waste Water Treatment Works (WwTWs)

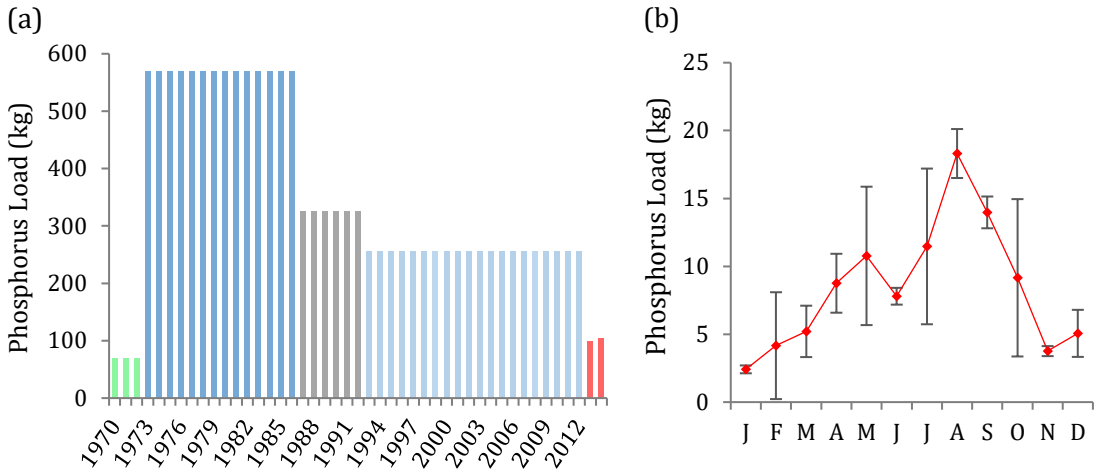

Figure A.2. (a) Annual WwTW phosphorus loads from 1970 to 2014 and (b) average monthly loads between 2013 and 2014. Error bars represent standard deviation of the mean.

1970 – 1972: During this period only Near Sawrey WwTW was operational, from which treated sewage effluent was piped to Esthwaite Water. Agar et al. (1988) estimated that the phosphorus load from this source was *c*. 70 kg y^{-1}. This load was apportioned between months according to the monthly apportionment of annual WwTW phosphorus loads in 2013 and 2014 (Figure A.2b).

1973 – 1986: Hawkshead WwTW was established in 1973. Agar et al. (1988) estimated that *c*. 410 kg y^{-1} of phosphorus was discharged to the main inflow of Esthwaite Water (Black Beck) in treated effluent. An additional phosphorus load of *c*. 90 kg y^{-1} was estimated to reach the lake due to the overflow of untreated wastewater during times of high rainfall. These loads were added to the Near Sawrey WwTW load of 70 kg y^{-1} and the total WwTW annual load was apportioned by month, as above.

1987 – 1992: Phosphorus stripping by chemical precipitation was added to the sewage treatment process at Hawkshead WwTW in 1986. This process reduced the Hawkshead WwTW phosphorus load to the lake by *c*. 60 % from 410 kg y^{-1} to *c*. 160 kg y^{-1} (Hall *et al.* 1993). Again, the annual Hawkshead WwTW, Near Sawrey WwTW and intermittent storm overflow loads were summed and apportioned by month.

1993 – 2012: Effluent for Near Sawrey WwTW was redirected to Cunsey Beck, below Esthwaite Water. The contribution of Near Sawrey WwTW (70 kg y^{-1}) was subtracted from the annual total WwTW phosphorus loads for this period.

2013 – 2014: During this period, the treated effluent from Hawkshead WwTW was sampled biweekly and analysed for TP concentration (TP_{WwTW}, µg l^{-1}), and United Utilities provided a record of daily mean effluent discharge (Q_{WwTW}, m^3 s^{-1}). Both variables were averaged by month for use in Equation 3.3 to calculate Hawkshead WwTW phosphorus load (Figure A.2b).

Extensive upgrades to Hawkshead WwTW were conducted between 2010 and 2012. The capacity of pumping stations and pre-treatment storage tanks was increased to eliminate the intermittent release of untreated wastewater to Black Beck during periods of heavy rainfall. As such, the 90 kg y^{-1} previously added to the annual total WwTW load was not applied to the 2013 and 2014 loads.

Fish Farm

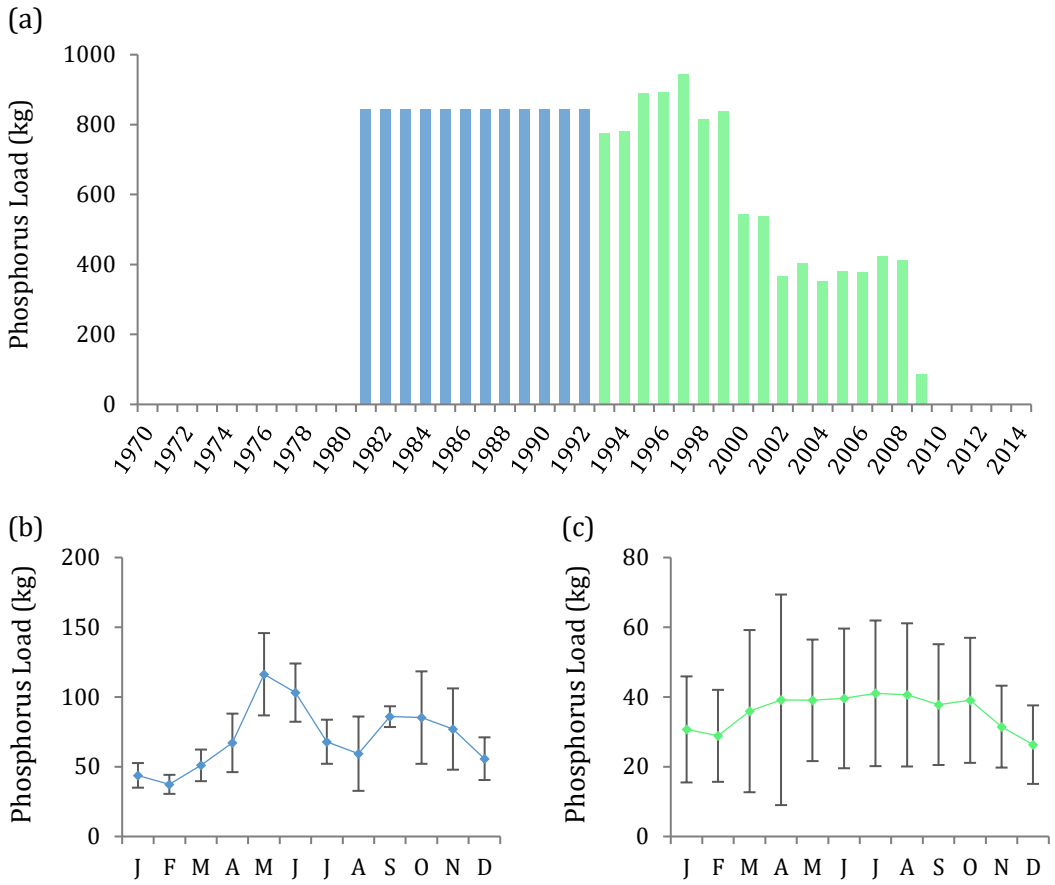

Figure A.3. (a) Annual fish farm phosphorus loads from 1970 to 2014 and average monthly loads between (b) 1993 and 1998 (inorganic), and (c) 1999 and 2009 (organic). Error bars represent standard deviation of the mean.

1970 – 1980: Prior to the installation of aquaculture cages to Esthwaite Water.

1981 – 1992: There is no record of the mass of fish food added to culture cages between 1981 and 1992 to use in calculations of fish farm phosphorus load. It was assumed that the food used during this period was an inorganic variety and so average monthly loads from 1993 to 1998 were used (Figure A.3b).

1993 – 2009: Esthwaite Water Trout fishery kept detailed records of the amount and type of fish food added to culture cages each month throughout this period. In calculations of fish farm phosphorus load the proportional phosphorus content of fish food (TP_{FF}, Equation 3.4) is dependent on the type of food used. In 1999, the farm switched from inorganic to organic fish food. The phosphorus contents of inorganic and organic food

from one of the most commonly used brands are 0.9 % and 1.6 %, respectively (Skettering). Therefore, a TP_{FF} value of 0.9 x 10^{-3} was used when calculating fish farm phosphorus loads before 1999, and a value of 1.6 x 10^{-3} used thereafter.

2010 – 2014: Cessation of fish farming in 2009 removed this source of phosphorus to Esthwaite Water.

Direct Rainfall

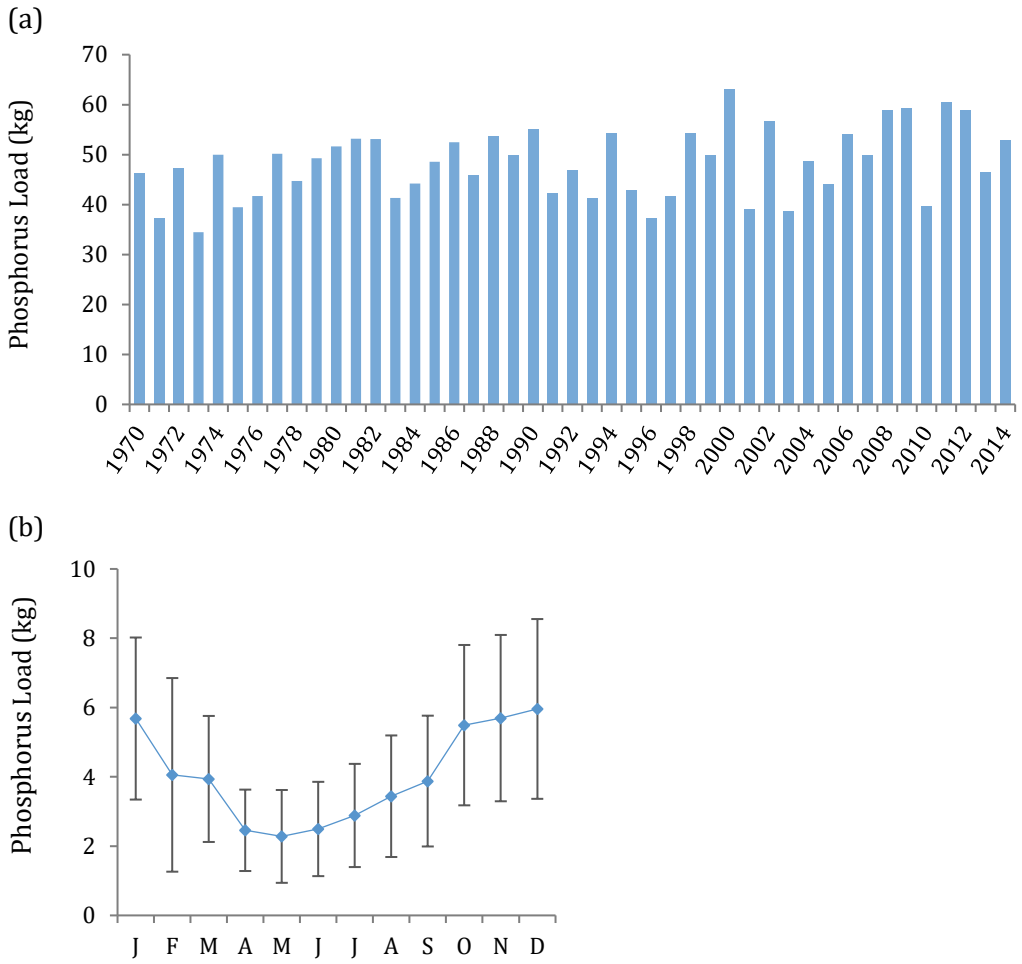

Figure A.4. (a) Annual rainfall phosphorus loads from 1970 to 2014 and (b) average monthly loads between 1970 and 2014. Error bars represent standard deviation of the mean.

1970 – 2014: Rainfall data (mm d^{-1}) was available for the whole study period. Rain water TP concentration (TP_{rain}) was not measured as part of this project. Previous nutrient budgeting attempts for Esthwaite Water have used a rain water TP concentration of 25 µg l^{-1} (May *et al.* 1997), which was the TP concentration of rain water collected in the Loch Leven catchment area, central Scotland (Bailey-Watts & Kirika 1987). In the absence of TP_{rain} data for a site closer to Esthwaite Water, the same concentration was used in calculations of rainfall phosphorus load in this study (Equation 3.6).

Outflow (Cunsey Beck)

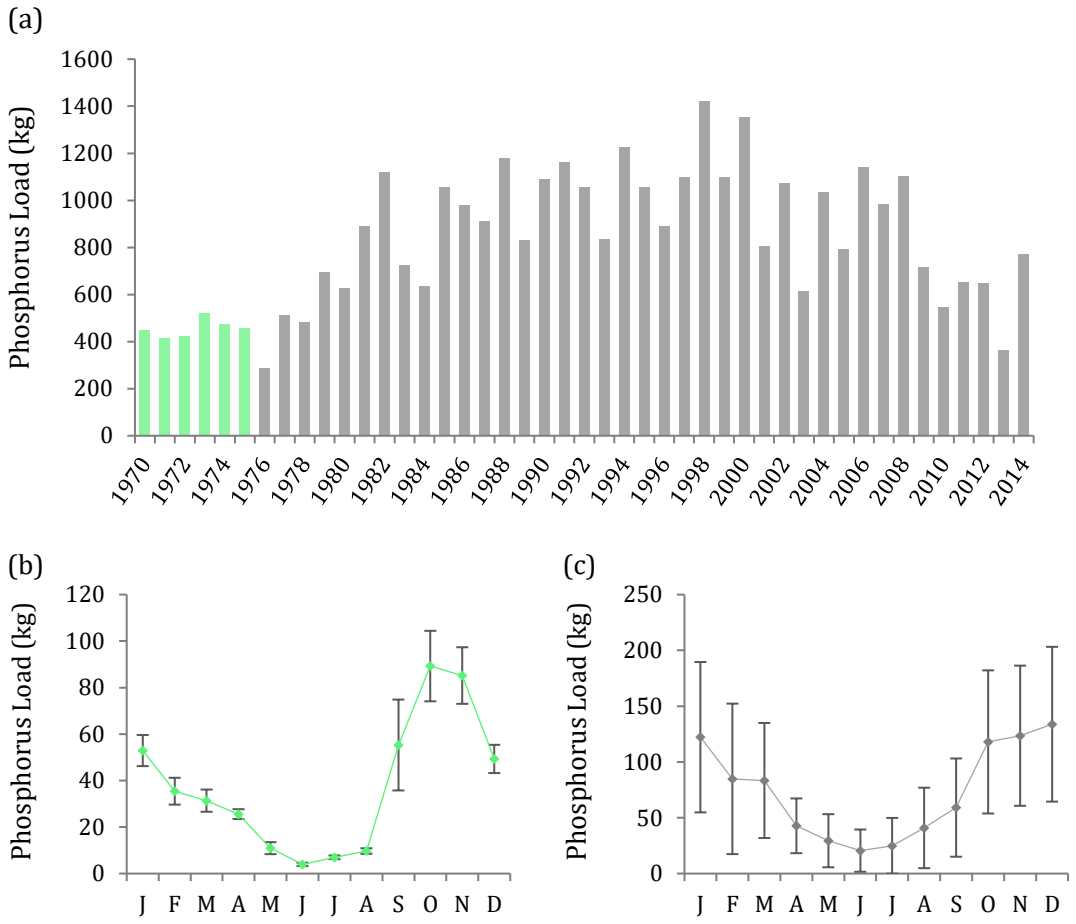

Figure A.5. (a) Annual outflow phosphorus loads from 1970 to 2014 and average monthly loads between (b) 1970 and 1975, and (c) 1976 and 2014. Error bars represent standard deviation of the mean.

1970 – 1975: The outflow (Cunsey Beck) phosphorus load was calculated using the same equation as used for the Black Beck phosphorus load (Equation 3.1). The TP concentration of the outflow was assumed to be the same as water column TP concentration. There is no discharge data for Cunsey Beck prior to 1976. The outlet was excavated in 1981, which markedly increased discharge. Average monthly discharges measured by the NRFA from 1976 until the time of excavation were adopted as the typical values for this period.

1976 – 2014: Discharge data were available from the NRFA for the whole of this period. Again, the TP concentration of Cunsey Beck at the lake outlet was assumed to be the same as the water column concentration.